Specification

A METHOD TO DIAGNOSE SCHIZOPHRENIA USING BLOOD

Background of the Invention

1. Field of the invention

[0001] The present invention relates to an objective method for diagnosis of schizophrenia using gene expression in blood as an index.

2. Prior art

[0002] Schizophrenia is a mental disorder and about 0.8% of the population suffers from schizophrenia during their youth. For it takes a long time to recover from schizophrenia, social loss caused by schizophrenia is immensely large. Therefore, enthusiastic investigations have been made in many laboratories all over the world to develop therapies and diagnoses for schizophrenia. In particular, significant progress has been made on therapies since development of dopamine receptor antagonists such as chlorpromazine. In contrast, diagnosis of schizophrenia is still classified based on psychological symptoms such as paranoid type, disorganized type, catatonic type and a type incapable to be classified, even in the latest US diagnostic reference "DSMIV". Therefore, diagnosis of schizophrenia finally relies upon subjective diagnosis made by the doctor in attendance, hence, diagnostic accuracy of schizophrenia has not been sufficient. Under such circumstances, chromosomal mapping of gene responsible for schizophrenia and identification of the gene have been made enthusiastically. However, definitive reports have been not made on such gene yet.

[0003] In occurrence of schizophrenia, it is known that considerable genetic backgrounds are underlying as risk factors. According to recent progress in genetic analysis, it is revealed that plural genes are involved in schizophrenia (Chiu YF et al., Mol Psychiatry, 2002; 7(6): 658-664; Reference 1). Moreover, from above-mentioned pathognomy, the disease itself is considered to be a complex disease comprising plural disorders (Kirkpatrick B et al., Arch Gen Psychiatry, 2001; 58(2): 165-171; Reference 2).

[0004] Recently, it has been recognized that schizophrenia is not only a local disease involved in cerebral nerve system, but a systemic disease involved in

immune system etc. Actually, it has been revealed that components of peripheral serum such as epidermal growth factor (Nawa H et al., Mol Psychiatry, 2002; 7(7): 673-682; Reference 3), brain-derived neurotrophic factor (Nawa H et al., Mol Psychiatry, 2002; 110(3): 249-257; Reference 4) and interleukin-8 (Akiyama et al., Schizophr Res., 1995; 37(1): 97-106; Reference 5) exhibit alteration in their expression levels in accordance with occurrence of the disease. Therefore, though patent properties on theses components have been acquired as a diagnostic method for the disease, the accuracy of determination remained to be low.

[0005] Moreover, as prior references concerning pathologic diagnosis by multi-measurement using a DNA micro-array or a DNA chip, references of following (6), (7) and (8) can be listed. In addition, literatures on analysis using brain tissue (not using peripheral blood like this invention) by a DNA micro-array or a DNA chip, references of following (9) and (10) can be listed. Furthermore, as prior arts involved in diagnosis of schizophrenia using proteins in blood, references of following (11), (12) and (13) can be listed. Furthermore, in addition to above-mentioned references, Patent Publication No.JP 2001-235470, Patent Publication JP 2001-245661, Patent Application JP 2000-331742, Patent Application JP 2001-228038 (Patent Publication JP 2003-038198) and Patent Application JP 2002-036937 (Patent Publication JP 2003-235557) can be listed.

[0006] Reference List

(1) Chiu YF et al., Mol Psychiatry, 2002; 7(6): 658-664.

(2) Kirkpatrick B et al., Arch Gen Psychiatry, 2001; 58(2): 165-171.

(3) Nawa H et al., Mol Psychiatry, 2002; 7(7): 673-682.

(4) Nawa H et al., Mol Psychiatry, 2002; 110(3): 249-257.

(5) Akiyama et al., Schizophr Res., 1995; 37(1): 97-106.

(6) Bertucci F, Viens P, Hingamp P, Nasser V, Houlgatte R, Birnbaum D., Int. J Cancer. 2003; 103(5): 565-571.

(7) Staudt LM.. N. Engl. J. Med., 2003; 348(18): 1777-1785

(8) Bertucci F, Houlgatte R, Nguyen C, Viens P, Jordan BR, Birnbaum D.,Lancet Oncol. 2001; 2(11): 674-682.

(9) Hakak, Y. et al., Proc Natl Acad Sci USA. 2001; 98: 4746-4751

(10)Mirnics, K. et al., Neuron. 2000; 28: 53-67.

(11)Toyooka, K. et al. Neurosci Res., 2003; 46: 299-307.
(12)Futamura, T. et al. Mol Psychiatry., 2002; 7: 673-682.
(13) Toyooka, K. et al. Psychiatry Res., 2002; 110: 249-257.

Summary of the Invention

[0007] The present invention was performed to solve aforementioned problems. An object of the present invention is to provide a reliable method for diagnosis of schizophrenia using small amount of blood as a sample, without forcing risk or pain to a patient.

[0008] To solve said problems, the present invention provides a method for diagnosing whether a test subject suffers from schizophrenia or not, the method comprising the steps of;

obtaining mononuclear cells in blood containing nucleic acid from said subject,

measuring the content of at least one nucleic acid selected from the group consisting of nucleic acid(s) (containing its fragment and a nucleic acid complementary to the nucleic acid) defining gene(s) exhibiting altered expression by occurrence of schizophrenia or nucleic acid(s) (containing its fragment and a nucleic acid complementary to the nucleic acid) defining gene(s) exhibiting altered expression by progression of schizophrenia in said mononuclear cells, and

determining alteration of the quantified level(s) of the gene(s) in said test subject is statistically significant in comparison with the quantified level(s) of said nucleic acid(s) defining gene(s) exhibiting altered expression by occurrence of schizophrenia or said nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia in healthy subjects or schizophrenic patients, thereby diagnosing whether said subject is suffering from schizophrenia or not.

[0009] Moreover, the present invention provides a method for diagnosing whether a test subject suffers from schizophrenia or not, the method comprising the steps of;

obtaining protein derived from mononuclear cells in blood from said test subject,

measuring the content of at least one protein selected from the group consisting of protein(s) (containing its fragment) encoded by nucleic acid(s)

defining gene(s) exhibiting altered expression by occurrence of schizophrenia or protein(s) (containing its fragment) encoded by nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia in said mononuclear cells, and

determining alteration of the quantified level of the protein(s) in said test subject is statistically significant in comparison with the quantified level(s) of said protein(s) encoded by nucleic acid(s) defining gene(s) exhibiting altered expression by occurrence of schizophrenia or said protein(s) encoded by nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia in healthy subjects or schizophrenic patients, thereby diagnosing whether said subject is suffering from schizophrenia or not.

[0010] According to the method of this invention, one can diagnose whether a test subject suffers from schizophrenia or not objectively without invasion. The method according to present invention provides a scientific and reliable method compared with conventional subjective methods for diagnosis of schizophrenia, and reinforces conventional diagnostic methods based on psychological symptoms.

[0011] Hereafter, the present invention is explained in detail. However, these detailed description of preferred embodiments and examples do not mean any restriction or limitation of the scope of the present invention.

Brief Description of the Drawings

[0012] Fig. 1 is a figure showing distribution of healthy subjects, chronic patients and acute patients in the Mahalanobis cluster analysis.

[0013] Fig. 2 is a graph showing the discrimination points analyzed by mRNA levels of kinases/phoaphatases.

[0014] Fig. 3 is a graph showing the result of Mahalanobis cluster analysis using kinases/phoaphatases.

Detailed Description of Preferred Embodiments

[0015] The present invention was achieved based on the knowledge obtained by the present inventors that the mRNA expression levels of 132 kinds of genes listed in Table 1 described below are altered by occurrence of schizophrenia with statistical significance. Moreover, mRNAs expression levels of 34 kinds of genes listed in Table 2 described below are altered by progression of

schizophrenia with statistical significance. As described in detail in the following examples, the present inventors found 152 genes exhibiting altered expression accompanied with this disease, by comparing the expression levels of about 12000 kinds of genes from peripheral mononuclear cells from acute and chronic schizophrenic patients in hospital with those from normal individuals. Moreover, statistical values measured on these genes are shown in Table 3 described below.

[0016] Moreover, among genes listed in Table 1, 24 genes exhibited altered expression in acute non-treated acute schizophrenic patients compared with normal individuals. Furthermore, 111 genes exhibited altered expression in chronic schizophrenic patients in hospital. Meanwhile, 3 genes exhibited altered expression in both patient groups redundantly.

[0017] Incidentally, "nucleic acid(s) defining gene(s) exhibiting altered expression by occurrence of schizophrenia" in this specification means nucleic acid(s) described in Table 1, that is defined by the gene name, the protein name which is a gene product and the nucleic acid sequence name, as well as GenBank Accession Nos. described with the names. Among the genes listed in Table 1, No.1 to No.99 and No.129 to No.132 are included in a group of genes exhibiting decreased expression by schizophrenia. Whereas, among the genes listed in Table 1, No.99 to No.128 are included in a group of genes exhibiting increased expression by schizophrenia.

[0018] Furthermore, "nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia" in this specification means nucleic acid(s) described in Table 2, that is defined by the gene name, the protein name which is s gene product and the nucleic acid sequence name, as well as GenBank Accession Nos. described with the names. As described in the following Example, the genes described in Table 1 and Table 2 consisting of 152 genes in total are useful for the purpose of the invention to diagnose schizophrenia. Meanwhile, statistical values of the 152 genes described in Tables 1 and 2 are disclosed in Table 3, and the genes attached with the mark "NS" in Table 3 are those exhibiting altered expression by progression of schizophrenia and corresponds to genes listed in Table 3.

[0019] The method according to this invention is also useful for diagnose

whether or not a test animal other than human, especially mammals, suffers from schizophrenia. In the following, a test animal means an animal other than human being, preferably mammals.

[0020] That is, this invention also provides a method for diagnosing whether a test animal suffers from schizophrenia or not, the method comprising the steps of;

obtaining mononuclear cells in blood containing nucleic acid from said test animal,

measuring the content of at least one nucleic acid selected from the group consisting of nucleic acid(s) (containing its fragment and a nucleic acid complementary to the nucleic acid) defining gene(s) exhibiting altered expression by occurrence of schizophrenia or nucleic acid(s) (containing its fragment and a nucleic acid complementary to the nucleic acid) defining gene(s) exhibiting altered expression by progression of schizophrenia in said mononuclear cells, and

determining alteration of the quantified level(s) of the gene(s) in said test animal is statistically significant in comparison with the quantified level(s) of said nucleic acid(s) defining gene(s) exhibiting altered expression by occurrence of schizophrenia or said nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia in healthy animals or schizophrenic animals, thereby diagnosing whether said animal is suffering from schizophrenia or not.

[0021] Moreover, this invention provides a method for diagnosing whether a test animal suffers from schizophrenia or not, the method comprising the steps of;

obtaining protein derived from mononuclear cells in blood from said test animal,

measuring the content of at least one protein selected from the group consisting of protein(s) (containing its fragment) encoded by nucleic acid(s) defining gene(s) exhibiting altered expression by occurrence of schizophrenia or protein(s) (containing its fragment) encoded by nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia in said mononuclear cells, and

determining alteration of the quantified level of the protein(s) in said test

animal is statistically significant in comparison with the quantified level(s) of said protein(s) encoded by nucleic acid(s) defining gene(s) exhibiting altered expression by occurrence of schizophrenia or said protein(s) encoded by nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia in healthy animals or schizophrenic animals, thereby diagnosing whether said animal is suffering from schizophrenia or not.

[0022] Table 1

No.	Genbank	Gene Name
1	AI677689	Homo sapiens cDNA, 3 end/clone=IMAGE-2329930, EST wd33c06.x1
2	Z23115	Bcl-Xl
3	X69115	ZNF37A mRNA for zinc finger
4	X07024	HSCCG1 Human X chromsome mRNA for CCG1 protein inv.in cell proliferation
5	L42243	Interferon receptor, alternatively spliced interferon receptor (IFNAR2)
6	HG960-HT960	Guanine Nucleotide Exchange Factor 1
7	Z12173	Glucosamine-6-sulphatase precursor
8	X98176	MACH-beta-1 protein (Caspase 8)
9	W25921	15a11 Homo sapiens cDNA /gb=W25921 /gi =1306044 /ug=Hs.164036 /len=723
10	Z35102	Ndr protein kinase
11	U28964	14-3-3 protein
12	X74262	RbAp48 mRNA encoding retinoblastoma binding protein
13	Y09568	SNAP23B protein
14	AF038960	SKD1 homolog
15	AI955897	Homo sapiens cDNA, 3 end/clone=IMAGE-2509049 ETS wt31b09.x1
16	X69086	Utrophin
17	M80629	Cdc2-related protein kinase (CHED)
18	U12022	Calmodulin type 1 (CALM1)
19	X74594	Rb2/p130 protein
20	M64174	Protein-tyrosine kinase JAK1
21	M28212	GTP-binding protein RAB6
22	U94333	Clq/MBL/SPA receptor C1qR(p)
23	U13948	Zinc finger/leucine zipper protein (AF10)
24	U96919	Inositol polyphosphate 4-phosphatase type I-beta
25	U26398	Inositol polyphosphate 4-phosphatase

No.	Genbank	Gene Name
26	AF068836	Cytohesin binding protein HE
27	L43821	CAS like protein for enhancer of filamentation (HEF1)
28	U17032	Rho GTPase activated protein type 5 (p190-B)
29	AB022017	AMP-activated protein kinase alpha-1
30	AF038897	Syntaxin 16
31	HG846-HT846	Cyclophilin-Related Protein
32	L04288	Natural killer-tumor recognition sequence
33	S66213	Integrin alpha 6B (CD49f)
34	AF052160	Homo sapiens clone 24629 mRNA sequence
35	AB015982	Protein kinase C Nu (EPK2), EPK2 mRNA for serine/threonine kinase
36	S79325	mRNA for SYT.SSX1 translocational target region of human synovial inducible sarcomas [Partial Mutant, 3' genes, 585nt],[synovial sarcomas, mRNA Partial Mutant, 3 genes, 585 nt]
37	M55536	Glucose transporter pseudogene
38	X97674	Nuclear receptor intermediary activation factor 2 (TIF2), transcriptional intermediary factor 2
39	U16028	CRE-BP1 transcription factor
40	M27504	Topoisomerase type II beta (Topo II)
41	U13044	Nuclear respiratory factor-2 subunit alpha
42	AC004990	PAC clone DJ1185I07 from 7q11.23-q21
43	AF048732	Cyclin T2b
44	AF061261	Zinc finger protein type C3H (MBLL)
45	U29671	MEK kinase (Mekk)
46	AB023967	Rod1
47	U07794	HSTXK Human tyrosine kinase (TXK)
48	U48736	Serine/threonine-protein kinase PRP4h (PRP4h)
49	AJ001810	Pre-mRNA cleavage factor I subunit Im
50	AI961669	Homo sapiens cDNA, 3 end/clone=IMAGE-2512364 EST wt65e11×1
51	Z48579	Disintegrin-metalloprotease
52	AF047432	ADP-ribosylation factor no.6 (ARF6)
53	U50553	Helicase like protein 2 containing DEAD/H box, helicase like protein 2 (DDX14)
54	U57317	P300/CBP-associated factor (P/CAF)
55	U08316	Ribosomal protein S6 kinase, insulin-stimulated protein kinase 1 (ISPK-1)
56	X77794	Cyclin G1
57	U43083	Guanine binding protein type q, G alpha-q (Gaq)

No.	Genbank	Gene Name
58	AF094481	Trinucleotide repeat CGG-DNA binding protein p20-CGGBP (CGGBP)
59	L12002	Integrin alpha 4 subunit
60	AB002450	Chromosome 5q21-22, clone-A3-A
61	X77723	Unknown protein of uterine endometrium
62	M97935	Transcription factor ISGF-3 (STAT91)
63	AC002086	Human PAC clone DJ525N14 from Xq23
64	AF100539	SH2 domain protein 1A isoform B (SH2D1A)
65	AJ001683	Killer cell lectin-like receptor NKG2F (NKG2F)
66	U13896	Human homolog of Drosophila discs gene, isoform 2 (hdlg-2)
67	U57452	Human SNF1-like protein kinase
68	X14798	Human DNA for c-ets-1 proto-oncogene
69	D16815	EAR-1r
70	L22075	Guanine nucleotide regulatory protein (G alpha 13)
71	L49229	Retinoblastoma susceptibility protein (RB1)
72	D13540	SH-PTP3 for protein-tyrosine phosphatase
73	W25874	EST 14e9 Homo sapiens cDNA
74	AF007111	MDM2-like p53-binding protein (MDMX)
75	AA013087	Homo sapiens cDNA, 5 end/clone=IMAGE-360208 EST ze27c09.r1
76	J04101	Erythroblastosis virus oncogene homolog 1 (ets-1)
77	X74837	HUMM9, Man9-mannosidase alpha, class 1A
78	X65873	Kinesin heavy chain 5B
79	U50648	Interferon-inducible RNA-dependent protein kinase (Pkr)
80	X15949	Interferon regulatory factor-2 (IRF-2)
81	AI189226	Homo sapiens cDNA, 3 end/clone=IMAGE-1722789 EST qd04h11.x1
82	D32039	Chondroitin sulfate proteoglycan PG-M (bursicon), proteoglycan PG-M(V3)
83	AL049962	Homo sapiens mRNA; cDNA DKFZp564P0823 (from clone DKFZp564P0823)
84	W27675	36b3 Homo sapiens cDNA
85	AW006742	Homo sapiens cDNA, 3 end/clone=IMAGE-2489058 EST wr28g10.x1
86	AA457029	Homo sapiens cDNA, 3 end/clone=IMAGE-815515 EST aa 38b10.s1
87	J03069	c-myc proto-oncogene (MYCL2)
88	Z24459	Mature T cell proliferation factor c6.1B gene; MTCP1 gene

No.	Genbank	Gene Name
89	AB018340	Homo sapiens mRNA for KIAA0797 protein
90	X02751	N-ras
91	U94747	WD repeat protein HAN11
92	AB028971	Homo sapiens mRNA for KIAA1048 protein
93	AB007923	Homo sapiens mRNA for KIAA0454 protein
94	AB026891	Cystine/glutamate transporter
95	U04735	Microsomal stress 70 protein ATPase core (stch)
96	X07767	cAMP-dependent protein kinase catalytic subunit type alpha (EC 2.7.1.37)
97	AA058762	Homo sapiens cDNA, 5 end/clone=IMAGE-487691
98	M69177	Monoamine oxidase B (MAOB)
99	M20560	Lipocortin-III (annexins A3)
100	AB007977	Homo sapiens chromosome 1 specific transcript KIAA0508
101	D10202	Platelet-activating factor receptor
102	AL048308	DKFZp586A2224_s1 Homo sapiens cDNA
103	X66363	PCTAIRE-1 for serine/threonine protein kinase
104	M94345	Gelsolin; macrophage capping protein; villin
105	W27466	31c9 Homo sapiens cDNA
106	Y15909	Diaphanous type 2 isoform 12C protein, dia-156 protein (DIA-156)
107	X02160	Insulin receptor precursor
108	L41827	Heregulin type 1; sensory and motor neuron-derived factor (HRG alpha)
109	AF026548	Branched chain alpha-ketoacid dehydrogenase kinase precursor (BCKD kinase)
110	X71129	Electron transfer flavoprotein beta subunit
111	U88153	P160
112	U08015	Calciceurin dependently activated T cell nuclear factor (NF-Atc)
113	AB011135	Homo sapiens mRNA for KIAA0563 protein
114	X13839	Vascular smooth muscle alpha-actin
115	AF076838	Rad17-like protein (RAD17)
116	AI762213	Homo sapiens cDNA, 3 end/clone=IMAGE-2394055 EST wi54d04.x1
117	L77213	Phosphomevalonate kinase
118	D17530	Drebrin E
119	D64109	Tob family transducer ERBB2,2
120	AB016816	MASL1
121	AB023211	Homo sapiens mRNA for KIAA0994 protein

No.	Genbank	Gene Name
122	AA521060	Homo sapiens cDNA, 3 end/clone=IMAGE-826408 EST aa71e09.s1
123	X77094	Neutrophil cytoplasmic factor type 4 (P40phox)
124	HG2689-HT2785	Mucin 5b, Tracheobronchial
125	AC004893	Homo sapiens PAC clone DJ0808A01 from 7q21.1-q31.1
126	AB028973	Homo sapiens mRNA for KIAA1050 protein
127	AI148772	Homo sapiens cDNA, 3 end/clone=IMAGE-1714897 EST qc69b01.x1
128	AL109724	Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 417629
129	L12691	Defensins alpha 3 (neutrophil peptide-3)
130	M34379	Elastase/medullasin
131	AF002224	Angelman Syndrome Gene, E6-AP ubiquitin protein ligase 3A (UBE3A)
132	X69089	Skeletal muscle 165kD protein

[0023] Table 2

No.	Genbank	Gene name
1	AI677689	Homo sapiens cDNA, 3 end/clone=IMAGE-2329930, EST wd33c06.x1
2	X74837	HUMM9,Man9-mannosidase alpha, class1A
3	D32039	Chondroitin sulfate proteoglycan PG-M (bursicon), proteoglycan PG-M(V3)
4	Z24459	Mature T cell proliferation factor c6.1B gene; MTCP1 gene
5	X07767	cAMP-dependent protein kinase catalytic subunit type alpha (EC 2.7.1.37)
6	AW003733	Homo sapiens cDNA, 3 end/clone=IMAGE-2497327
7	M20560	Lipocortin-III (annexins A3)
8	X66363	PCTAIRE-1 for serine/threonine protein kinase
9	AI762213	Homo sapiens cDNA, 3 end/clone=IMAGE-2394055 EST wi54d04.x1
10	AA522537	Homo sapiens cDNA, 3 end/clone=IMAGE-979142 EST ni38e08.s1
11	U66359	Human T54 protein (T54)
12	Z80345	acyl-CoA dehydrogenase; SCAD gene
13	D17530	Drebrin E
14	L36645	Receptor protein-tyrosine kinase EphA4 (HEK8)
15	D64109	Tob family transducer BRBB2,2
16	AI039144	Homo sapiens cDNA, 3 end/clone=IMAGE-1657913 EST ox31b09.s1
17	AF000573	Homogentisate 1,2-dioxygenase
18	AB016816	MASL1
19	AA528252	Homo sapiens cDNA, 3 end/clone=IMAGE-965972 EST nh92c11.s1
20	M14648	Cell adhesion protein (vitronectin) receptor alpha subunit (CD51)
21	AL049435	Cluster Incl AL049435:Homo sapiens mRNA; cDNA DKFZp586B0220 (from clone DKFZp586B0220)
22	L40392	Homo sapiens (clone S164) mRNA, 3 end of cds /cds
23	AB023226	Homo sapiens mRNA for KIAA1009 protein
24	AB018259	Homo sapiens mRNA for KIAA0716 protein
25	AJ132099	Vanin-like gene; vnn1 gene; VNN1 protein
26	AC004893	Homo sapiens PAC clone DJ0808A01 from 7q21.1-q31.1

No.	Genbank	Gene name
27	AF001549	Human Chromosome 16 BAC clone CIT987SK-A-270G1
28	X55544	Transcriptional factor TREB protein
29	AB011120	Homo sapiens mRNA for KIAA0548 protein
30	U01877	P300; transcriptional adaptor protein; E1A-binding protein
31	L25851	Integrin alpha E precursor (CD103)
32	AI148772	Homo sapiens cDNA, 3 end/clone=IMAGE-1714897 EST qc69h01.x1
33	L12691	Defensins alpha 3 (neutrophil peptide-3)
34	AL036554	Homo sapiens cDNA, 5 end/clone=DKFZp564J2262·1

[0024] Table 3

No.	Genbank		Healthy subject group		Threshold of healthy subject group		Patient group		Threshold of patient group		Total of patient group	Welch T test				
			Mean	S.D.	5% threshold	1% threshold	Mean vaue	Standard deviation	5% threshold	1% threshold	Ratio of mean value	P Comparison with acute patient group	Signifi-cance	P Comparison with chronic patient group	Signifi-cance	
1	AI677689	decrease	1.12	0.81	-0.18	-0.76	0.92	0.61	1.90	2.33	0.82	0.049	CN	0.947		NS
2	Z23115	decrease	1.72	0.93	0.23	-0.44	0.82	0.79	2.08	2.63	0.47	0.027	CN	0.051		
3	X69115	decrease	2.19	1.20	0.27	-0.60	1.07	1.18	2.96	3.78	0.49	0.404		0.02	CS	
4	X07024	decrease	1.64	0.74	0.46	-0.07	0.76	0.44	1.47	1.78	0.47	0.043	CN	0.008	CS	
5	L42243	decrease	1.61	0.62	0.62	0.17	0.83	0.35	1.39	1.63	0.51	0.044		0.003	CS	
6	HG960-HT960	decrease	1.53	0.48	0.75	0.40	0.79	0.48	1.55	1.89	0.52	0.082		0.001	CS	
7	Z12173	decrease	1.97	0.77	0.75	0.19	0.85	0.60	1.82	2.24	0.43	0.184		0.001	CS	
8	X98176	decrease	1.83	0.58	0.91	0.49	0.80	0.53	1.64	2.01	0.44	0.056		0	CS	
9	W25921	decrease	1.48	0.47	0.74	0.40	0.81	0.43	1.51	1.81	0.55	0.209		0.001	CS	
10	Z35102	decrease	1.53	0.36	0.95	0.69	0.80	0.36	1.37	1.63	0.52	0.048		0	CS	
11	U28964	decrease	1.57	0.51	0.75	0.38	0.84	0.33	1.36	1.59	0.53	0.046		0.001	CS	
12	X74262	decrease	1.47	0.45	0.75	0.43	0.83	0.39	1.45	1.72	0.56	0.196		0.001	CS	
13	Y09568	decrease	1.56	0.41	0.91	0.62	0.86	0.41	1.51	1.79	0.55	0.163		0	CS	
14	AF038960	decrease	1.61	0.60	0.66	0.22	0.83	0.48	1.61	1.95	0.52	0.247		0.001	CS	
15	AI955897	decrease	1.88	0.64	0.85	0.39	1.00	0.52	1.83	2.19	0.53	0.255		0.001	CS	
16	X69086	decrease	1.64	0.62	0.65	0.21	0.91	0.44	1.61	1.92	0.56	0.276		0.003	CS	
17	M80629	decrease	1.72	0.57	0.81	0.40	0.92	0.43	1.61	1.90	0.54	0.215		0.001	CS	
18	U12022	decrease	1.61	0.48	0.84	0.49	0.80	0.36	1.38	1.63	0.50	0.041		0	CS	
19	X74594	decrease	1.77	0.47	1.02	0.68	0.91	0.48	1.68	2.01	0.51	0.1		0	CS	
20	M64174	decrease	1.76	0.46	1.01	0.68	0.92	0.40	1.56	1.84	0.52	0.058		0	CS	
21	M28212	decrease	1.72	0.43	1.04	0.73	0.87	0.46	1.61	1.94	0.51	0.137		0	CS	
22	U94333	decrease	1.49	0.44	0.79	0.48	0.81	0.47	1.56	1.89	0.55	0.331		0	CS	
23	U13948	decrease	1.41	0.29	0.95	0.74	0.80	0.37	1.38	1.64	0.56	0.07		0	CS	
24	U96919	decrease	1.72	0.86	0.34	-0.28	0.86	0.46	1.61	1.93	0.50	0.198		0.008	CS	
25	U26398	decrease	1.39	0.47	0.64	0.31	0.70	0.42	1.38	1.67	0.51	0.037		0.001	CS	
26	AF068836	decrease	1.66	0.53	0.81	0.43	0.88	0.49	1.68	2.02	0.53	0.093		0.002	CS	
27	L43821	decrease	1.74	0.61	0.77	0.33	1.02	0.49	1.80	2.14	0.58	0.421		0.002	CS	
28	U17032	decrease	1.63	0.79	0.37	-0.20	0.94	0.61	1.91	2.34	0.58	0.497		0.014	CS	
29	AB022017	decrease	1.90	0.99	0.32	-0.39	0.74	0.51	1.55	1.91	0.39	0.029	CN	0.006	CS	
30	AF038897	decrease	1.97	0.75	0.77	0.23	0.80	0.57	1.71	2.10	0.41	0.083		0.001	CS	

No.	Genbank		Healthy subject group		Threshold of healthy subject group		Patient group		Threshold of patient group		Total of patient group	Welch T test				
			Mean	S.D.	5% threshold	1% threshold	Mean vaue	Standard deviation	5% threshold	1% threshold	Ratio of mean value	P Comparison with acute patient group	Signifi-cance	P Comparison with chronic patient group	Signifi-cance	
31	HG846-HT846	decrease	1.92	0.88	0.51	-0.12	0.84	0.54	1.70	2.08	0.43	0.152		0.003	CS	
32	L04288	decrease	2.39	1.48	0.02	-1.05	0.92	0.85	2.28	2.88	0.39	0.117		0.017	CS	
33	S66213	decrease	2.08	1.14	0.25	-0.57	0.93	0.54	1.79	2.17	0.45	0.134		0.009	CS	
34	AF052160	decrease	1.86	1.14	0.03	-0.79	0.91	0.65	1.95	2.40	0.49	0.222		0.026	CS	
35	AB015982	decrease	1.64	0.64	0.62	0.16	0.91	0.58	1.84	2.25	0.55	0.418		0.003	CS	
36	S79325	decrease	3.05	1.47	0.70	-0.35	0.92	1.13	2.72	3.51	0.30	0.232		0.001	CS	
37	M55536	decrease	2.89	1.96	-0.25	-1.67	0.88	1.16	2.74	3.56	0.30	0.274		0.007	CS	
38	X97674	decrease	2.64	1.56	0.15	-0.98	0.91	0.98	2.47	3.15	0.34	0.238		0.005	CS	
39	U16028	decrease	1.93	1.04	0.26	-0.49	0.85	0.70	1.96	2.45	0.44	0.3		0.007	CS	
40	M27504	decrease	2.47	1.43	0.18	-0.84	1.01	1.03	2.66	3.38	0.41	0.425		0.006	CS	
41	U13044	decrease	2.47	1.72	-0.29	-1.53	1.14	0.87	2.53	3.14	0.46	0.478		0.022	CS	
42	AC004990	decrease	2.57	1.17	0.70	-0.14	0.88	1.06	2.57	3.32	0.34	0.276		0.001	CS	
43	AF048732	decrease	2.36	1.33	0.23	-0.72	0.97	0.77	2.20	2.74	0.41	0.22		0.006	CS	
44	AF061261	decrease	1.95	1.14	0.12	-0.70	0.87	0.74	2.06	2.59	0.45	0.384		0.01	CS	
45	U29671	decrease	2.18	1.19	0.28	-0.58	1.06	0.81	2.36	2.93	0.49	0.438		0.01	CS	
46	AB023967	decrease	2.07	1.34	-0.07	-1.04	1.11	0.63	2.12	2.56	0.53	0.354		0.039	CS	
47	U07794	decrease	1.78	0.90	0.34	-0.31	0.94	0.71	2.07	2.57	0.53	0.49		0.01	CS	
48	U48736	decrease	1.94	1.20	0.02	-0.85	1.01	0.74	2.19	2.71	0.52	0.57		0.02	CS	
49	AJ001810	decrease	1.70	0.88	0.29	-0.35	0.98	0.72	2.14	2.64	0.58	0.943		0.009	CS	
50	AI961669	decrease	1.73	0.89	0.31	-0.33	0.93	0.57	1.84	2.24	0.54	0.514		0.009	CS	
51	Z48579	decrease	1.76	0.92	0.29	-0.38	0.77	0.65	1.81	2.27	0.44	0.259		0.005	CS	
52	AF047432	decrease	1.82	0.78	0.58	0.01	0.84	0.70	1.95	2.44	0.46	0.298		0.002	CS	
53	U50553	decrease	1.61	0.63	0.59	0.14	0.92	0.69	2.03	2.51	0.57	0.554		0.005	CS	
54	U57317	decrease	2.30	1.12	0.51	-0.30	0.97	0.72	2.12	2.62	0.42	0.144		0.005	CS	
55	U08316	decrease	1.91	0.94	0.40	-0.28	0.89	0.47	1.64	1.97	0.47	0.12		0.007	CS	
56	X77794	decrease	1.82	0.77	0.60	0.05	1.01	0.58	1.93	2.34	0.55	0.348		0.005	CS	
57	U43083	decrease	1.94	0.90	0.50	-0.15	0.91	0.53	1.76	2.13	0.47	0.193		0.004	CS	
58	AF094481	decrease	1.63	0.68	0.54	0.05	0.92	0.41	1.58	1.86	0.57	0.243		0.005	CS	
59	L12002	decrease	1.70	0.85	0.34	-0.28	0.86	0.44	1.57	1.88	0.51	0.195		0.009	CS	

No.	Genbank		Healthy subject group		Threshold of healthy subject group		Patient group		Threshold of patient group		Total of patient group	Welch T test				
			Mean	S.D.	5% threshold	1% threshold	Mean vaue	Standard deviation	5% threshold	1% threshold	Ratio of mean value	P Comparison with acute patient group	Signifi-cance	P Comparison with chronic patient group	Signifi-cance	
60	AB002450	decrease	1.81	1.17	-0.06	-0.90	0.93	0.64	1.96	2.41	0.51	0.316		0.037	CS	
61	X77723	decrease	1.81	0.72	0.66	0.14	0.93	0.79	2.19	2.74	0.51	0.593		0.001	CS	
62	M97935	decrease	2.69	1.76	-0.12	-1.39	1.06	0.99	2.65	3.34	0.39	0.321		0.012	CS	
63	AC002086	decrease	2.08	1.07	0.36	-0.41	0.85	0.58	1.78	2.18	0.41	0.139		0.004	CS	
64	AF100539	decrease	1.89	0.88	0.48	-0.15	0.90	0.59	1.85	2.26	0.48	0.092		0.007	CS	
65	AJ001683	decrease	1.86	0.82	0.54	-0.05	1.07	1.01	2.67	3.38	0.57	0.756		0.007	CS	
66	U13896	decrease	1.71	0.78	0.45	-0.11	0.96	0.87	2.35	2.96	0.56	0.61		0.012	CS	
67	U57452	decrease	3.12	2.13	-0.29	-1.83	0.95	0.65	1.98	2.44	0.30	0.041	CN	0.013	CS	
68	X14798	decrease	2.40	1.46	0.07	-0.98	0.92	0.63	1.93	2.38	0.38	0.11		0.01	CS	
69	D16815	decrease	2.36	1.40	0.11	-0.90	1.01	0.83	2.33	2.91	0.43	0.274		0.011	CS	
70	L22075	decrease	4.79	4.19	-1.92	-4.94	0.87	1.40	3.10	4.08	0.18	0.1		0.016	CS	
71	L49229	decrease	3.39	2.63	-0.82	-2.72	1.10	1.78	3.94	5.19	0.32	0.44		0.016	CS	
72	D13540	decrease	3.60	2.27	-0.02	-1.65	0.99	2.09	4.34	5.81	0.27	0.399		0.004	CS	
73	W25874	decrease	2.38	1.33	0.25	-0.71	0.70	0.99	2.28	2.98	0.29	0.146		0.002	CS	
74	AF007111	decrease	2.10	1.22	0.15	-0.73	1.00	0.96	2.54	3.21	0.48	0.424		0.016	CS	
75	AA013087	decrease	2.94	1.18	1.05	0.20	0.65	0.99	2.24	2.93	0.22	0.115		0	CS	
76	J04101	decrease	3.05	1.70	0.33	-0.90	0.72	1.04	2.38	3.11	0.24	0.089		0.001	CS	
77	X74837	decrease	1.93	1.09	0.18	-0.61	0.79	0.48	1.56	1.90	0.41	0.144		0.006	CS	NS
78	X65873	decrease	1.80	0.63	0.80	0.34	0.79	0.53	1.63	2.00	0.44	0.054		0	CS	
79	U50648	decrease	1.68	0.73	0.51	-0.02	0.86	0.35	1.42	1.66	0.51	0.113		0.004	CS	
80	X15949	decrease	1.50	0.55	0.63	0.23	0.79	0.39	1.42	1.70	0.53	0.095		0.003	CS	
81	AI189226	decrease	1.70	0.93	0.20	-0.47	0.85	0.43	1.54	1.84	0.50	0.047		0.027	CS	
82	D32039	decrease	1.30	0.38	0.69	0.42	0.83	0.58	1.76	2.16	0.64	0.878		0.002	CS	NS
83	AL049962	decrease	1.46	0.51	0.63	0.26	0.83	0.47	1.57	1.90	0.57	0.346		0.002	CS	
84	W27675	decrease	1.34	0.24	0.96	0.79	0.65	0.53	1.49	1.86	0.48	0.131		0	CS	
85	AW006742	decrease	1.50	0.41	0.85	0.55	0.77	0.55	1.64	2.03	0.51	0.157		0.001	CS	

No.	Genbank		Healthy subject group		Threshold of healthy subject group		Patient group		Threshold of patient group		Total of patient group	Welch T test				
			Mean	S.D.	5% threshold	1% threshold	Mean vaue	Standard deviation	5% threshold	1% threshold	Ratio of mean value	P Comparison with acute patient group	Signifi-cance	P Comparison with chronic patient group	Signifi-cance	
86	AA457029	decrease	1.36	0.34	0.81	0.56	0.80	0.42	1.47	1.77	0.59	0.386		0	CS	
87	J03069	decrease	1.43	0.47	0.68	0.33	0.86	0.50	1.67	2.02	0.60	0.492		0.002	CS	
88	Z24459	decrease	1.43	0.30	0.95	0.74	0.87	0.38	1.49	1.76	0.61	0.675		0	CS	NS
89	AB018340	decrease	1.13	0.17	0.85	0.73	0.72	0.40	1.36	1.63	0.64	0.89		0	CS	
90	X02751	decrease	1.75	0.97	0.19	-0.51	0.81	0.72	1.96	2.46	0.46	0.186		0.017	CS	
91	U94747	decrease	1.44	0.79	0.17	-0.40	0.72	0.50	1.52	1.88	0.50	0.084		0.025	CS	
92	AB028971	decrease	1.22	0.24	0.84	0.67	0.72	0.47	1.47	1.80	0.59	0.025	CN	0.014		
93	AB007923	decrease	1.54	0.56	0.64	0.23	0.82	0.41	1.47	1.75	0.53	0.027		0.003	CS	
94	AB026891	decrease	1.73	0.99	0.14	-0.58	0.96	0.60	1.92	2.34	0.55	0.4		0.028	CS	
95	U04735	decrease	1.70	0.63	0.70	0.25	0.95	0.43	1.63	1.93	0.56	0.164		0.003	CS	
96	X07767	decrease	1.38	0.76	0.16	-0.39	0.83	0.70	1.94	2.42	0.60	0.908		0.031	CS	NS
97	AW003733	decrease	1.08	0.36	0.51	0.25	0.84	0.63	1.85	2.29	0.77	0.147		0.037		NS
98	AA058762	decrease	1.38	0.73	0.21	-0.31	0.66	0.63	1.67	2.12	0.47	0.202		0.017	CS	
99	M69177	decrease	3.57	2.74	-0.81	-2.79	1.32	1.83	4.25	5.53	0.37	0.35		0.025	CS	
100	M20560	increaase	1.97	1.48	4.34	5.41	2.20	2.70	-2.12	-4.01	1.11	0.037	CN	0.391		NS
101	AB007977	increaase	0.69	0.73	-1.33	-1.00	1.65	1.14	-0.18	-0.97	2.39	0.566		0.007	CS	
102	D10202	increaase	0.58	0.65	-1.37	-0.93	2.09	1.67	-0.58	-1.74	3.60	0.12		0.011	CS	
103	AL048308	increaase	0.66	0.39	-0.39	-0.25	1.24	0.61	0.27	-0.15	1.89	0.362		0.003	CS	
104	X66363	increaase	0.34	0.64	2.14	-1.15	2.32	1.98	-0.84	-2.23	6.86	0.271		0.001	CS	NS
105	M94345	increaase	0.78	0.28	-0.23	0.13	1.30	0.48	0.53	0.20	1.66	0.035	CN	0.015		
106	W27466	increaase	0.76	0.24	-0.19	0.20	1.28	0.43	0.60	0.30	1.69	0.003	CN	0.012		
107	Y15909	increaase	0.68	0.39	-0.37	-0.22	1.27	0.45	0.55	0.24	1.87	0.022	CN	0.008		
108	X02160	increaase	0.57	0.42	-0.49	-0.40	1.02	0.29	0.56	0.36	1.78	0.002	CN	0.037		
109	L41827	increase	0.70	0.37	-0.35	-0.16	1.40	0.61	0.42	-0.01	2.00	0.008	CN	0.011		
110	AF026548	increaase	0.54	0.41	-0.49	-0.41	1.28	0.51	0.47	0.12	2.36	0.054		0.001	CS	
111	X71129	increaase	0.70	0.44	-0.46	-0.33	1.45	0.80	0.18	-0.38	2.09	0.144		0.008	CS	
112	U88153	increaase	0.70	0.41	-0.41	-0.26	1.28	0.65	0.24	-0.22	1.84	0.211		0.009	CS	
113	U08015	increase	0.80	0.24	-0.19	0.25	1.62	0.83	0.29	-0.29	2.02	0.078		0.007	CS	
114	AB011135	increase	0.71	0.34	-0.31	-0.09	1.27	0.58	0.34	-0.07	1.79	0.44		0.002	CS	
115	X13839	increase	0.65	0.41	-0.42	-0.30	1.53	0.81	0.24	-0.33	2.36	0.239		0.001	CS	

No.	Genbank		Healthy subject group		Threshold of healthy subject group		Patient group		Threshold of patient group		Total of patient group	Welch T test				
			Mean	S.D.	5% threshold	1% threshold	Mean vaue	Standard deviation	5% threshold	1% threshold	Ratio of mean value	P Comparison with acute patient group	Signifi-cance	P Comparison with chronic patient group	Signifi-cance	
116	AF076838	increaase	0.65	0.53	-0.66	-0.57	1.37	0.67	0.31	-0.16	2.10	0.127		0.005	CS	
117	AI762213	increaase	0.81	0.22	-0.16	0.31	1.35	0.86	-0.03	-0.63	1.66	0.391		0.006	CS	NS
118	AA522537	increaase	0.89	0.42	-0.38	-0.10	1.35	0.94	-0.15	-0.81	1.51	0.6		0.052		NS
119	U66359	increaase	0.76	0.42	-0.40	-0.21	1.00	0.54	0.13	-0.25	1.30	0.18		0.06		NS
120	Z80345	increaase	1.08	1.03	-1.60	-1.32	1.06	0.60	0.11	-0.31	0.98	0.121		0.575		NS
121	L77213	increaase	0.68	0.55	-0.69	-0.59	1.26	0.96	-0.28	-0.96	1.86	0.016	CN	0.168		
122	D17530	increaase	1.05	0.62	-0.62	-0.39	0.89	0.64	-0.13	-0.58	0.85	0.015	CN	0.848		NS
123	L36645	increaase	0.78	0.46	-0.46	-0.29	1.06	0.55	0.17	-0.21	1.36	0.473		0.043		NS
124	D64109	increaase	0.60	0.52	-0.71	-0.61	1.41	1.11	-0.36	-1.14	2.35	0.771		0.01	CS	NS
125	AI039144	increaase	1.11	0.82	-0.95	-0.79	1.40	1.29	-0.67	-1.57	1.26	0.188		0.241		NS
126	AF000573	increaase	1.27	0.72	-0.70	-0.41	1.33	1.02	-0.30	-1.02	1.05	0.046		0.426		NS
127	AB016816	increaase	0.66	0.78	-1.90	-1.16	1.54	1.66	-1.12	-2.29	2.34	0.205		0.019	CS	NS
128	AB023211	increaase	0.72	0.34	-0.30	-0.06	1.29	0.80	0.01	-0.55	1.79	0.044	CN	0.149		
129	AA521060	increaase	0.99	0.31	-0.24	0.28	1.37	0.68	0.28	-0.19	1.38	0.005	CN	0.565		
130	X77094	increaase	0.90	0.41	-0.36	-0.05	1.64	1.11	-0.13	-0.91	1.83	0.035	CN	0.179		
131	HG2689-HT27	increaase	0.72	0.48	-0.51	-0.39	1.32	0.72	0.17	-0.33	1.84	0.005	CN	0.135		
132	AA528252	increaase	1.15	0.48	-0.41	0.02	1.07	0.62	0.08	-0.36	0.93	0.013		0.161		NS
133	M14648	increaase	1.07	0.42	-0.35	0.09	1.02	0.63	0.01	-0.42	0.95	0.08		0.064		NS
134	AL049435	increaase	1.07	0.34	-0.26	0.29	1.15	0.64	0.13	-0.32	1.07	0.036		0.119		NS
135	L40392	increaase	1.13	0.22	-0.16	0.61	1.05	0.64	0.02	-0.43	0.93	0.08		0.008		NS
136	AB023226	increaase	1.05	0.41	-0.34	0.09	0.96	0.61	-0.01	-0.43	0.92	0.044		0.106		NS
137	AB018259	increaase	0.84	0.69	-0.90	-0.77	1.03	0.52	0.20	-0.17	1.23	0.012		0.791		NS
138	AJ132099	increaase	1.22	0.54	-0.47	-0.04	1.08	0.72	-0.07	-0.57	0.88	0.016		0.027		NS
139	AC004893	increaase	0.88	0.29	-0.23	0.21	1.16	0.70	0.04	-0.45	1.31	0.034	CN	0.977		NS
140	AB028973	increase	0.73	0.47	-0.49	-0.36	1.15	0.52	0.32	-0.05	1.57	0.013	CN	0.225		
141	AF001549	increaase	1.04	0.58	-0.55	-0.30	1.02	0.78	-0.22	-0.77	0.98	0.135		0.294		NS
142	X55544	increaase	1.05	0.42	-0.35	0.06	0.89	0.54	0.01	-0.37	0.85	0.11		0.082		NS
143	AB011120	increaase	0.96	0.40	-0.34	0.04	0.97	0.65	-0.07	-0.52	1.01	0.005		0.117		NS
144	U01877	increaase	1.28	0.94	-1.08	-0.89	1.36	0.99	-0.23	-0.93	1.06	0.095		0.492		NS
145	L25851	increaase	1.05	0.40	-0.33	0.12	1.10	0.80	-0.17	-0.73	1.05	0.001		0.477		NS

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			Mean	S.D.	5% threshold	1% threshold	Mean vaue	Standard deviation	5% threshold	1% threshold	Ratio of mean value	P Comparison with acute patient group	Signifi-cance	P Comparison with chronic patient group	Signifi-cance	
146	AI148772	increaase	0.70	0.54	-0.65	-0.55	1.23	1.00	-0.37	-1.08	1.75	0.006	CN	0.891		NS
147	AL109724	increaase	0.79	0.73	-1.09	-0.91	1.45	1.13	-0.37	-1.16	1.84	0.003	CN	0.316		
148	L12691	decrease	1.64	1.07	-0.08	-0.85	1.84	2.30	5.52	7.13	1.12	0.007	CN	0.325		NS
149	AL036554	decrease	1.26	0.82	-0.05	-0.64	1.72	2.30	5.39	7.00	1.36	0.016		0.202		NS
150	M34379	decrease	1.71	1.01	0.08	-0.65	2.25	3.42	7.72	10.12	1.32	0.007	CN	0.278		
151	AF002224	decrease	9.96	11.12	-7.83	-15.84	2.53	6.68	13.22	17.90	0.25	0.613		0.04	CS	
152	X69089	decrease	5.03	4.83	-2.70	-6.18	1.87	2.35	5.63	7.27	0.37	0.578		0.041	CS	
			253.80	77.84			161.02	54.81								

[0025] It was determined that the genes listed in Table 1 are particularly useful as an index for diagnosis of schizophrenia, in consideration of all of the following criteria on a DNA chip:

(1) having reliable signal intensity,

(2) exhibiting gene-expression alteration ratio of more than two-folds or less than half, wherein the gene-expression alteration ratio is determined by either one of "comparison of the average expression levels between the non-treated acute patient group and the healthy subject group" or "comparison of the average expression levels between the chronic patient group in hospital and the healthy subject group", (3) exhibiting p-value of 0.05 or less obtained from test of difference in the average level of gene expression between the patient group and the normal group. Note that the term "p-value" is the probability of measuring a certain statistical value according to null hypothesis. Therefore Table 1 consists of sum of the list of genes obtained by two kinds of statistic comparison as follows: (1) comparison of the average expression levels of genes between the non-treated acute patient group and the healthy subject group, and (2) comparison of the average expression levels of genes between the chronic patient group in hospital and the healthy subject group. Therefore, when the patient group was defined as the sum of the non-treated acute patient group and the chronic patient group in hospital, comparison between the average expression levels of genes in the patient group and that in the healthy subject group does not necessarily result in the p value (significance in probability) of less than 0.05.

[0026] It was shown that the genes listed in Table 2 can satidfiy all of the following criteria on a DNA chip:

(1) having reliable signal intensity,

(2) exhibiting gene-expression alteration ratio of more than two-folds or less than half, wherein the gene-expression alteration ratio is determined by "comparison of the average expression levels between the non-treated acute patient group the chronic patient group in hospital",

(3) exhibiting p-value of 0.05 or less obtained from test of difference in the average level of gene expression, when the non-treated acute patient group was compared with the chronic patient group in hospital. The genes exhibit alteration with progression of schizophrenia, and thus assumed to reflect the

pathology of schizophrenia itself. Therefore, when only the average expression levels of genes were compared between the patient group and the healthy subjects, the comparison does not necessarily result in the p value of less than 0.05.

[0027] However, for the purpose to improve accuracy of diagnosis and to discriminate the acute phase and chronic phase of schizophrenia (in details, refer to the following Example) the "gene(s) exhibiting altered expression by progression of schizophrenia " can be selected for the purpose of this diagnosis, then the accuracy of diagnosis can be increased. The genes and proteins to be used as the index may be selected from these genes or proteins, in the simplest may be selected based upon the p value alone or the gene-expression alteration ratio alone, otherwise may be selected based upon the standard deviation of the normal group, theta of non-treated acute patient group or that of chronic patients group in hospital.

[0028] When the index gene is selected on the basis of p value alone as the criteria, the index gene may preferably have the p value of 0.2 or less, 0.15 or less, more preferably 0.10 or less and more 0.05 or less. Further preferably, the index gene may have the p value of 0.02 or less, 0.01 or less, 0.005 or less, 0.025 or less, 0.002 or less, 0.001 or less.

[0029] When the index gene is selected on the basis of the gene-expression alteration ratio alone as the criteria, the index gene may have the gene-expression alteration ratio of 2.0 or more, preferably the gene-expression alteration ratio of 2.1 or more, 2.2 or more, 2.25 or more, 2.5 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 7.5 or more, 8 or more, 9 or more, 10 or more.

[0030] Moreover, the method of the present invention can be utilized for the purpose to diagnose objectively whether a test subject suffers from schizophrenia or not, using the expression of the gene or fragment thereof and/or the protein encoded by the gene or fragment thereof satisfying the aforementioned criteria.

[0031] According to this specification, the term "schizophrenia" includes any type of schizophrenia such as paranoid schizophrenia, disorganized schizophrenia, catatonic schizophrenia, and a type of schizophrenia incapable to be classified. As to genes involved in schizophrenia, genetic polymorphism of these genes has been known. Therefore, by using a DNA chip, a method capable of capturing

expression of larger number of gene is identical, then a method to diagnose "schizophrenia" based upon plural criteria on gene expression is developed as described in this specification. Thus among genes listed in Table 1 and Table 2 described above, gene expression may measured on one gene, preferably 2 genes, more preferably 5 genes, 10 genes, 20 genes, 30 genes, 50 genes, 100 genes, and determination may be performed comprehensively.

[0032] According to the method of the present invention, at least one nucleic acid or its fragment and/or at least one protein or its fragment encoded by the nucleic acid selected from the group consisting of the genes represented by the Genbank accession no. described in Table 1 and Table 2 may be quantified. In some cases, plural protein names or plural Genbank accession nos. may be registered or become to be popular for one gene, a nucleic acid specified as such is also included within the scope of this invention.

[0033] The "nucleic acid(s) complementary to the nucleic acid(s) defining gene(s) exhibiting altered expression by occurrence of schizophrenia" typically means mRNAs and cDNAs of genes represented by the Genbank accession no. described in Table 1. Moreover, the "nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia" typically means mRNAs and cDNAs of genes represented by the Genbank accession no. described in Table 2. Furthermore, any polynucleotides, such as regulatory sequences or polyadenyl sequences, may be included in the terminal ends of the translation region and/or inside of these mRNA or cDNA.

[0034] The "fragment of a nucleic acid defining gene(s) exhibiting altered expression by occurrence of schizophrenia" means a polynucleotide consisting of a part of nucleic acid(s) defining gene(s) represented by the Genbank accession no. described in Table 1 while retaining its biological function. Typically, it may be a restriction fragment of a mRNA or a cDNA corresponding to the gene represented by the Genbank accession no. described in Table 1. In the same manner, the "fragment of a protein encoded by a nucleic acid(s) defining gene(s) exhibiting altered expression by occurrence of schizophrenia" means a polypeptide consisting of a part of protein(s) encoded by the nucleic acid(s) defining gene represented by the Genbank accession no. described in Table 1 while retaining its biological function.

[0035] Moreover, the "fragment of a nucleic acid defining gene(s) exhibiting altered expression by progression of schizophrenia" means a polynucleotide consisting of a part of nucleic acid(s) defining gene(s) represented by the Genbank accession no. described in Table 2 while retaining its biological function. Typically, it may be a restriction fragment of a mRNA or a cDNA corresponding to the gene represented by the Genbank accession no. described in Table 2. In the same manner, the "fragment of a protein encoded by a nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia" means a polypeptide consisting of a part of protein(s) encoded by the nucleic acid(s) defining gene represented by the Genbank accession no. described in Table 2 while retaining its biological function.

[0036] The term "nucleic acid" used in this specification may include any polynucleotide consisting of simple nucleotides and/or modified nucleotides such as cDNA, mRNA, total RNA and hnRNA. The term "modified nucleotides" may include phosphoric esters such as inosine, acetylcytidine, methylcytidine, methyladenosine and methyl guanosine, as well as other postnatal nucleotides which may be produced by the effect of ultraviolet rays or chemical substances.

[0037] Mononuclear cell in blood is also called monocyte, which is a cell having single nuclear in blood and corresponds to large lymphocyte. Macrophage in inflammatory sites is included herein and such vell exhibits strong phagocytic effect. Erythrocyte may removed from total blood cell fraction treated by anti-coagulant under non-isotonic condition, and blood cell fraction thus obtained may be purified by classification according to cell volume using sucrose density-gradient centrifugation method or ficoll centrifugation method.

[0038] In general, to achieve quantification of nucleic acids, a sample may be obtained from a test subject, which is succeeded by extraction of ribonucleic acid from the sample. Extraction of the nucleic acid from a component of living body may be achieved by any extraction method such as phenol extraction and ethanol precipitation. To achieve extraction of mRNA, the sample may be passed through an oligo-dT column.

[0039] In the case where the amount of the nucleic acid is not large, the nucleic acid may be amplified, if necessary. The nucleic acid may be amplified

by polymerase chain reaction (hereinafter, simply referred to as "PCR"), for example, by reverse transcriptase PCR (RT-PCR). Furthermore, as described in the following description, the amplification may be performed as a quantitative operation or the quantitative operation may be combined with other operations.

[0040] After the extraction procedure and/or the amplification procedure (if necessary) may be achieved, at least one nucleic acid or fragment thereof selected from the group consisting of nucleic acids defining genes represented by GenBank Accession nos. listed in Table 1 or 2, may be quantified. Otherwise, at least one protein or fragment thereof encoded by the nucleic acid may be quantified.

[0041] The nucleic acid may be quantified by any conventional known method, such as quantitative PCR, Northern blotting, RNase protection mapping, or a combination of such methods. In the case the kinds of genes to be quantified is small, these methods of quantitative PCR, Northern blotting, RNase protection mapping may be effective. In the Example, a DNA chip is utilized, however, above-mentioned procedures are simpler and lower in price. However, the embodiment of the present invention is not limited to that utilizing a DNA chip.

[0042] The internal nucleotides of the amplified products may be labeled in the quantitative PCR, typically by using radio-labeled nucleotides (e.g.,^{32}P). Alternatively, the amplified product may be endo-labeled by using radio-labeled primers. Free radio-labeled nucleotides or radio-labeled primers may be separated from the labeled amplified products, by using some known methods including gel filtration, alcohol precipitation, trichloroacetic acid precipitation and physical absorption using a glass filter. Thereafter, procedures such as electrophoresis and hybridization may be performed (or may not be performed) and the amplified products may be quantified by using liquid scintillation, autoradiography, and imaging plate Bio-Imaging Analyzer (BAS; Fuji Photo Film Co., Ltd.). Instead of such radioactive substance, a fluorescent substance or a luminescent substance may be used as a labeling substance, and the amplified product may be quantified by means of spectrofluorometer, fluoromicro plate reader or CCD camera. Furthermore, in the case where incorporation of the labeling substance into the amplified product is not performed during the PCR operation, an intercalate fluorescent pigment such as ethidium bromide, SYBR

Green I^{TM}, PicoGreen^{TM} (manufactured and sold by Molecular Probes) may be used to detect the amplified product.

[0043] In the case where the quantitative PCR may not be performed, the sample containing nucleic acid may be subjected to electrophoresis, and then analyzed by Southern blotting or Northern blotting, thereafter quantification may be achieved by using a probe labeled with a detectable marker.

[0044] In the case where many kinds of nucleic acids are to be quantified simultaneously, DNA chip or DNA microarray may be used together with or instead of the aforementioned techniques. In this case, many kinds of nucleic acids can be quantified and analyzed at one procedure, such means is useful for multiplication of probabilities and for linear discriminant analysis. Moreover, a DNA chip or a DNA micro-array on which the "nucleic acid(s) defining gene(s) exhibiting altered expression by progression of schizophrenia" is immobilized may be prepared for the purpose to be applied in diagnosis of schizophrenia and a kit may be also prepared. Various methods can be adopted to prepare a DNA or a DNA micro-array on which a nucleic acid is immobilized, such methods are well known to those skilled in the art. In concrete, DNA may be arranged onto a substrate at extremely high density and the arranged DNA may be analyzed, otherwise, DNA may be synthesized directly on a substrate. Arrangement of DNA on a substrate at extremely high density may be achieved by utilizing a commercially available spotter.

[0045] Instead of quantification of the nucleic acid or together with quantification of the nucleic acid, the amount of gene expression may be indirectly determined by quantifying the amount of protein produced from the nucleic acid (gene). When schizophrenia is diagnosed according to the method of the present invention, in many cases, the indirect method of quantifying protein(s) encoded by nucleic acid(s) may be more useful than the direct method of quantifying nucleic acid(s). Therefore, in the case a nucleic acid is to be quantified indirectly by quantification of a protein, it is preferred to investigate on the extent of the alteration of the protein to be quantified observed in a schizophrenic patient in comparison with healthy subjects (non-mental patient).

[0046] For the method of protein extraction from cells and for the method of protein quantification, any methods known in this field may be used.

The proteins (gene products) produced in many blood cells are released into blood in many cases, therefore, proteins in blood, plasma or serum can be used as a sample of a test subject. Examples of methods for protein quantification may include Western blotting method and enzyme-linked immunosorbent assay method including solid-phase enzyme-linked immunosorbent assay, immunocytochemistry, and immunohistochemistry. Alternatively, in the case an antibody toward the target protein is available, cells may be labeled by fluorescence by means of immunocytochemistry and the fluorescent intensity of the cells may be quantified by cell sorter, thereby determination may be achieved.

[0047] Meanwhile, only summary of the conventional procedures are schematically exemplified in this specification, however, such description should not be interpret to limit the range of this invention. Therefore, modified or alternative methods of the aforementioned methods can be also utilized.

[0048] Extraction, amplification, isolation, and quantification of the nucleic acid can be performed automatically by using an automatic operation device currently on the market, in which an electrophoresis device and a PCR device and the like are combined, therefore, utilization of such device may be preferred. By using such an automatic machine, diagnosis of schizophrenia can be achieved in the same manner as routine clinical tests.

[0049] After quantification of a prescribed nucleic acid, whether a test subject suffers from schizophrenia or not may be determined, using the quantified value as the index. In the case diagnosis is made by using quantitative value of a singular nucleic acid as the index, the threshold value may be determined appropriately with reference to a normal value. Then, if the quantified value is higher or lower than the threshold value, it is highly possible that the test subject suffers from schizophrenia. In the group of genes listed in Table 1, the genes described in No.1 to No.98 and No.129 to No.132 exhibit decreased expression by occurrence of schizophrenia. Therefore, if the quantified value is lower than the predetermined threshold of healthy subjects, the test subject can be diagnosed to be schizophrenic at high probability.

[0050] Meanwhile, in the group of genes listed in Table 1, the genes described in No.99 to No.128 exhibit increased expression by occurrence of schizophrenia. Therefore, if the quantified value is higher than the

predetermined threshold of healthy subjects, the test subject can be diagnosed to be schizophrenic at high probability. Moreover, in the group of genes shown in Table 3, 24 genes attached with "CN" mark are included in a gene group obtained by the comparison between the healthy subjects and non-treated patients, whereas 111 genes attached with "CS" mark are included in a gene group obtained by comparison between healthy subjects and chronic patients

[0051] Conversely, as to genes described in No.1 to No.98 and No.129 to No.132 of Table 1, when the quantified values of these nucleic acids obtained from a test subject are higher than the known threshold of overall schizophrenic group, the test subject is not included in the patient group. That is, the test subject is diagnosed to be "normal". In the same manner, as to genes described in No.99 to No.128 of Table 1, when the quantified values of these nucleic acids obtained from a test subject are lower than the known threshold of acute and chronic schizophrenic groups, the test subject is not included in the patient group. That is, the test subject is diagnosed to be "normal".

[0052] At present, schizophrenia is considered to be a syndrome consisting of plural diseases (paranoid type schizophrenia, disorganized type, catatonic type schizophrenia and a type of schizophrenia incapable to be classified). Therefore, it is desired to diagnose in comprehensive manner by combination of plural genes applicable for determination of schizophrenia at high probability, rather than diagnosis based upon only singular gene.

[0053] The threshold value may be selected depending upon the accurately of the diagnosis required, as shown below.

[0054] When distribution on the amount of gene expression is elucidated on both the non-schizophrenic group (hereinafter, referred to as normal group) and the schizophrenic group (hereinafter, simply referred to as patient group) groups, the upper threshold or the lower threshold may be selected in such a manner that an individual (from which the nucleic acids to be determined have been obtained) belongs to the normal group with probability of 10%, 5%, or 1%.

[0055] When distribution on the amount of gene-expression is elucidated for only the normal group, it can be hypothesized that an individual (from which the nucleic acids to be determined have been obtained) belongs to the normal group. Under this hypothesis, the threshold (the amount or concentration of nucleic

acid) may be determined so as to such quantified value can be obtained with a probability (hereinafter, referred to as p-value, one-sided probability may be also utilized for the direction of the alteration is known in advance) of 10%, preferably 5%, more preferably 1%. For example, null hypothesis can be verified using the value of the gene expression level obtained from a DNA chip, in accordance with the 5% threshold value or the 1% threshold value represented in Table 3, that is; the sample having the determined value is included in the normal group or patient group.

[0056] In a method for determination of nucleic acid not using a DNA chip, when a part of genes or plural genes described in Table 1 and Table 2 are adopted to determine whether a subject is "normal" or "abnormal", the distribution and variance of the gene may preferably be investigated on healthy subjects and schizophrenic patients in advance. In that case, when distribution of the gene expression has been elucidated only one of the healthy subject group and the patient group, analysis can be made using identical statistical method.
The p-value can be calculated by a statistical method such as t-test or non-parametric test.

[0057] To elucidate statistical distribution of gene-expression on the normal group and/or the patient group, it is generally required that at least 5 individuals, preferably 10 individuals, more preferably 20 individuals, more preferably 30 individuals, further preferably 50 individuals and the most preferably 100 individuals are to be measured.

[0058] Moreover, depending on the correlation with schizophrenia or reliability of the determination, the plural genes selected may be treated by addition at different weights, and the multiplication or mathematical conversion may be performed on the values of expression levels of individual genes to increase the accuracy of determination. It is also possible to determine whether a test subject suffers from schizophrenia or not with higher accurately by using arbitrary statistic methods of various kinds, thus a diagnostic method using such statistic methods should be included within the scope of the present invention.

[0059] In the case where diagnosis is made using the quantified value of a singular nucleic acid as the index, as described in detail in the following Examples, the singular nucleic acid may preferably satisfy following criteria; (1)

the amount of expression in either of the normal group or the patient group is high (2) the absolute gene-expression alteration ratio between the normal group and patient group is 2.0 or more (refer to "Examples"), and (3) the p-value in the test of mean-values difference is 5% or less.

[0060] In the case where diagnosis is made using the quantified values of plural nucleic acids, appropriate thresholds should be determined on each of the nucleic acids. Then diagnosis can be made in the same manner when a singular nucleic acid is used as the index, by examining whether the amount of gene expression is higher or lower than the threshold for individual genes.

[0061] If one quantified value of the nucleic acid(s) is higher or lower than the threshold in accordance with the accuracy required, the test subject may be diagnosed to be schizophrenic. If more than two quantified values of the nucleic acid(s) are higher or lower than the thresholds, the test subject may be diagnosed to be schizophrenic at higher possibility. In the case confirmed diagnosis is required, when more numbers of the quantified values of nucleic acids are above or below compared with the threshold, the diagnosis of schizophrenia can be made with more accurately. It is also possible to make determination using the amount of expression of these plural genes through statistic calculation such as mathematical formula, multiplication of probabilities and weighted linear addition.

[0062] The diagnostic method of the present invention can be used together with the conventional subjective diagnostic method. Moreover, if quantified data on the amount of nucleic acid(s) can be collected from patients clearly suffering from schizophrenia (determined by some means) and such data is applicable as the index for the diagnostic method of the present invention, it is possible to make confirmed diagnosis by the method of the present invention alone.

[0063] The subject of the present invention is to provide a method for objective diagnosis for schizophrenia, therefore, not to provide particular individual procedures for extraction, amplification and analysis described concretely in this specification. Hence, it should be noted that diagnostic method utilizing other than above-mentioned procedures are also include in the scope of present invention.

[0064] As described in the above, according to the method of the present invention, objective diagnosis can be made on whether a test subject suffers from schizophrenia or not, by using the amount of expression of nucleic acid (gene) as an index.

[0065] Moreover, the method of this invention is also useful for diagnosis of transition of schizophrenia; i.e. from acute phase to chronic phase of schizophrenia. In concrete, the genes described in Table 2 are included in a group of genes exhibiting difference in the expression between the acute patient group and the chronic patient group, therefore, these genes reflect pathologic alteration of schizophrenia. Therefore, using the genes listed in Table 2 as an index, objective diagnosis can be made on whether the pathology of a test subject is at the acute phase of schizophrenia or the pathology of test subject is changing to the chronic schizophrenia.

[0066] The diagnostic method of the present invention can be applied to a psychiatric assessment for the purpose to examine whether a subject is legally responsible or not, and to a psychiatric assessment performed for other purposes.

[0067] Furthermore, the method of this invention can be utilized in development of a medicine for treatment of schizophrenia. In such case, a candidate compound to be screened may be administrated to a model animal of schizophrenia. If the model animal recovers from schizophrenia, it may be determined that said candidate substance is effective as an anti-schizophrenic agent. In concrete, for example, when the quantified level of the nucleic acid(s) defining gene(s) exhibiting decreased expression by schizophrenia (Table 1, No.1-98 and No.129-132) approaches to the control level with significance compared with the level of the nucleic acid(s) measured prior to administration of said candidate compound, said candidate compound may be effective as an anti-schizophrenic agent. For example, if the level of the nucleic acid(s) defining gene(s) exhibiting altered expression with significance in non-treated acute patients described in Table 3 or gene products thereof is quantified and the quantified level approaches to the normal level with respect to a chronic patient in hospital who received treatment a medicine, such alteration the quantified value(s) of the gene(s) is assumed to reflect the effect of the medicine.
In concrete, No.1 (Homo sapiens cDNA, 3 end/clone=IMAGE-2329930 EST

wd33c06.x1: Genbank No. AI677689), No.100 (lipocortin-III: Genbank No. M20560), No.146 (Homo sapiens cDNA, 3 end/clone=IMAGE-1714897 EST qc69h01.x1: Genbank No. AI1487729), No.139 (Homo sapiens PAC clone DJ0808A01 from 7q21.1-q31.1: Genbank No. AC004893) and defensin alpha 3: Genbank No. L12691) are included within such gene.

[0068] Furthermore in the genes listed in Table 1 or Table 2, determination of the expression levels of genes (the amount of mRNA) involved in protein phosphorylation or de-phosphorylation is particularly useful in diagnosis of schizophrenia. In concrete, by linear discriminant analysis on the expression levels of the genes encoding following 16 kinds of proteins, it is possible to discriminate schizophrenic patients from healthy subjects; (1) Ndr protein kinase (Genbank No.Z35102), (2) protein-tyrosine kinase JAK1 (Genbank No.M64174), (3) inositol polyphosphate 4-phosphatase type I-beta (Genbank No.U96919), (4) AMP-activated protein kinase alpha-1 (Genbank No.AB022017), (5) Protein C kinase Nu (EPK2) : EPK2 mRNA for serine/threonine kinase (Genbank No.AB015982), (6) MEK kinase (Mekk) (Genbank No.U29671), (7) HSTXK Human tyrosine kinase (TXK) (Genbank No.U07794), (8) serine/threonine-protein kinase PRP4h (PRP4h) (Genbank No.U48736), (9) ribosome protein S6 kinase, insulin-stimulated protein kinase 1 (ISPK-1) (Genbank No.U08316), (10) Human SNF1-like protein kinase (Genbank No.U57452), (11) SH-PTP3 for protein-tyrosine phosphatase (Genbank No.D13540), (12) interferon-inducible RNA-dependent protein kinase (Pkr) (Genbank No.U50648), (13) cAMP-dependent protein kinase catalytic subunit type alpha (EC 2.7.1.37) (Genbank No.X07767), (14) PCTAIRE-1 for serine/threonine protein kinase (Genbank No.X66363), (15) branched chain alpha-ketoacid dehydrogenase kinase precursor (BCKD kinase) (Genbank No.AF026548) and (16) Phosphomevalonate kinase (Genbank No.L77213).

[0069] The levels of these genes are not altered in depressive patients and in panic disorders compared with control, then the diagnostic method according to this method is effective to discriminate schizophrenia from these other psychiatric diseases. Moreover, in the embodiment where plural genes are combined in the diagnosis, above-mentioned 16 genes may preferably be included within the target genes to be measured for the purpose to achieve higher accuracy.

[0070] Now, the present invention will be further explained in detail with reference to Experimental Examples, which will not limits the scope of the present invention in any sense.

Examples

[0071] In this Embodiment, explanation will be provided on the genes identified by the present inventors and available as a marker for diagnosis of schizophrenia.

[0072] In this study, blood sample was obtained from (1) acute schizophrenic patients (non-treated, samples N1 to N5), (2) chronic schizophrenia patients in hospital (treated by medicine, samples S1 to S12), and (3) normal volunteers without psychiatric disease (samples C1 to C9), in the presence of anticoagulant using a Venoject vacuum blood collection tube. Then mononuclear cells were separated and purified from the blood and RNA was extracted using an Isogen nucleic acid extraction kit (Nippon Gene).

[0073] According to the protocol provided by Affynmetrix Co., cDNA was synthesized by transcriptase using an oligo dT primer with T7 promoter, and double strand DNA was prepared using E.coli DNA polymerase. The DNA was purified, and cRNA was transcribed by T7 RNA synthase using a biotin UTP as a substrate. The obtained cRNA was fragmented by the treatment with a solution containing magnesium acetate and potassium acetate. A 30 μg of the cRNA was hybridized with Genechip-U95A (version 2, Affymetrix), then expression of the gene was measured all together and patterning (molecular expression profiling) was performed. The hybridization signal was visualized by fluorescence using avidin/R-Phycoerythrin. The signal was read with a fluorescent reader (Gene array scanner G2500A, Hewlett-Packard). Signals from gene spots were analyzed using a Microarray Suite program (Affymetrix). The signal intensities were determined from the sum of the signals from perfect matches (PM) oligo probes, by subtracting the sum of signals from miss match (MM) oligo probes. To compensate for the variation among different DNA chips, the result was calibrated by representing the values as the ratios toward the median of the positive genes on each of the DNA chips to give normalized signal intensities.

[0074] To confirm genes exhibiting widespread alteration in plural schizophrenic patients, two comparisons were performed separately, i.e. the comparison between the acute schizophrenic group (samples N1 to N5) and the

normal volunteer group (samples C1 to C9), the comparison between the chronic schizophrenic group (samples S1 to S12) and the normal volunteer group (samples C1 to C9). Table 4 shows the data obtained from the normal volunteers C1-C9. Table 4-1 shows the results from the individual subjects, and Table 4-2 shows averages and standard deviations (S.D.) obtained from the data. Table 5 shows the data from non-treated patients N1-N5 and data from treated chronic patients S1-S12. Table 5-1 shows the values on the non-treated patients, Table 5-2 shows the values on the treated patients respectively, and Table 5-3 shows averages and standard deviations obtained from the data.

[0075] Table 4—1

Genbank	Individual Gene No.	Healthy subject C1	Healthy subject C2	Healthy subject C3	Healthy subject C4	Healthy subject C5	Healthy subject C6	Healthy subject C7	Healthy subject C8	Healthy subject C9
U26398	25	1.01	1.25	1.27	0.99	2.33	1.83	1.61	1.40	0.85
J04101	76	0.37	4.55	1.09	4.95	3.33	5.32	2.83	2.96	2.05
AW006742	85	1.71	1.36	1.01	1.73	1.82	1.57	2.02	0.73	1.59
AB028971	92	1.05	1.60	0.99	1.23	1.26	1.49	1.33	0.83	1.24
AW003733	97	1.36	0.82	1.65	1.02	0.45	1.44	1.05	0.97	0.97
D10202	102	0.50	10.00	1.12	6.54	3.10	2.88	10.00	0.90	2.53
L41827	109	1.05	1.61	1.14	7.85	0.75	2.85	1.08	2.65	1.37
U08015	113	1.21	1.32	1.53	0.85	3.18	1.01	1.03	1.33	1.24
AB028973	140	0.90	2.74	2.67	1.53	6.77	1.79	1.10	1.33	0.59
AL036554	149	0.36	1.54	0.00	1.46	2.65	1.00	0.77	1.60	1.99
AF002224	151	1.29	1.90	12.03	4.89	26.43	27.89	15.20	0.00	0.00
		-7.00	4.00	3.00	3	7.00	7.00	-1.00	-3.00	0.00

[0076] Table 4-2

Genbank	Mean	Standard Deviation
U26398	1.39	0.47
J04101	3.05	1.70
AW006742	1.50	0.41
AB028971	1.22	0.24
AW003733	1.08	0.36
D10202	1.73	1.53
L41827	1.43	2.69
U08015	1.24	4.15
AB028973	1.37	2.13
AL036554	1.26	0.82
AF002224	9.96	11.12

[0077] Table 5—1

Genbank	Individual Gene No.	Non-treated patient N1	Non-treated patient N2	Non-treated patient N3	Non-treated patient N4	Non-treated patient N5
U26398	25	0.67	1.31	1.05	0.93	0.73
J04101	76	1.57	3.16	0.00	2.45	0.12
AW006742	85	1.76	1.52	0.71	0.34	0.79
AB028971	92	1.01	0.00	0.79	0.83	0.14
AW003733	97	1.59	1.60	1.37	0.83	1.50
D10202	102	0.40	3.71	0.35	0.44	2.31
L41827	109	0.62	1.21	0.53	0.62	0.74
U08015	113	0.56	1.49	0.44	0.61	1.00
AB028973	140	0.92	0.90	0.60	0.64	0.46
AL036554	149	0.00	0.57	0.00	1.00	0.00
AF002224	151	5.52	27.27	0.00	0.00	0.00
		−7.00	1	−9.00	−11.00	−9.00

[0078] Table 5—2

Genbank	Individual Gene No.	Chronic treated patient S1	Chronic treated patient S2	Chronic treated patient S3	Chronic treated patient S4	Chronic treated patient S5	Chronic treated patient S6	Chronic treated patient S7	Chronic treated patient S8	Chronic treated patient S9	Chronic treated patient S10	Chronic treated patient S11	Chronic treated patient S12
U26398	25	0.42	0.80	1.20	0.00	1.06	0.46	0.00	0.00	1.08	0.51	0.71	1.05
J04101	76	0.00	0.00	0.24	0.00	0.00	0.91	0.00	0.00	2.30	1.15	0.00	0.36
AW006742	85	1.09	0.57	1.65	0.29	0.99	0.14	0.21	0.90	0.36	0.15	0.31	1.35
AB028971	92	0.32	0.71	0.62	0.57	0.18	1.37	1.10	1.06	0.81	1.12	1.57	0.00
AW003733	97	0.08	0.98	1.06	0.78	0.06	0.00	0.00	0.19	0.15	1.09	1.70	1.24
D10202	102	0.32	0.26	0.47	4.80	0.28	0.76	0.39	0.16	0.39	1.61	10.00	1.25
L41827	109	0.66	0.61	0.36	1.14	0.95	1.16	0.58	0.59	0.45	2.45	0.84	2.00
U08015	113	0.67	0.27	0.36	0.49	1.22	1.00	1.12	0.91	0.91	1.10	0.55	0.38
AB028973	140	2.89	0.70	0.61	0.74	0.83	2.02	1.23	1.22	1.67	0.71	0.66	3.19
AL036554	149	3.80	0.00	3.74	1.33	7.19	0.00	6.82	0.72	0.85	1.36	1.14	0.71
AF002224	151	1.37	0.00	2.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.46
		−9.00	−11.00	−9.00	−9.00	−9.00	−7.00	−9.00	−11.00	−9.00	−3.00	−5.00	−5.00

[0079] Table 5−3

Genbank	Mean	Standard deviation	Ratio of Mean value
U26398	0.70	0.42	0.51
J04101	0.72	1.04	0.24
AW006742	0.77	0.55	0.51
AB028971	0.72	0.47	0.59
AW003733	0.84	0.63	0.77
D10202	0.48	0.60	0.28
L41827	0.72	1.63	0.50
U08015	0.62	1.20	0.50
AB028973	0.87	1.92	0.64
AL036554	1.72	2.30	1.36
AF002224	2.53	6.68	0.25

[0080] In this Example, the genes described in Table 1 were obtained by screening 12,000 genes that satisfy following three criteria:

(1) having reliable signal intensity by PRESENCE Call on DNA chip,

(2) exhibiting gene-expression alteration ratio of more than two-folds or less than half, wherein the gene-expression alteration ratio is determined by either one of "comparison between the average expression level of the non-treated acute patient group and the average expression level of the healthy subjects", "comparison between average expression level of the chronic patient group in hospital and the average expression level of the healthy subjects", or "comparison between the average expression level of the non-treated acute patient group and the average expression level of the chronic patient group in hospital", and

(3) exhibiting p-value of 0.05 or less when the difference on the average gene expression level is examined by Welch's t-test for significant difference on the following groups; the difference between the patient group (either one of the non-treated group (CN) or treated chronic group (CS)) and the normal group, or the difference between the non-treated acute patient group and the treated chronic group (NS). Twenty-four genes were identified by comparing the average expression levels between the non-treated acute patient group and the normal group; 111 genes were identified by comparing the average expression levels between the treated chronic patient group and the normal group; and 34 genes were identified by

comparing the average expression levels between the non-treated acute patient group and the treated chronic patient group. These genes exhibited significant alteration by acute or chronic schizophrenia.

[0081] Accordingly, these genes described in Tables 1 and 2 selected according to the above criteria are particularly useful as an index for diagnosis of schizophrenia. The data on the averages, distributions and probabilities (described in Table 3) obtained from the DNA chips of the following Examples are useful for creating criteria for practical diagnosis of schizophrenia.

Example 1: Singular determination

[0082] In this Example, description will be given about a diagnostic method for schizophrenia based upon one gene, using peripheral blood from a patient and adopting the expression level of mRNA for erythroblastosis virus oncogene homolog 1 (ETS-1) protein (v-ets avian erythroblastosis virus E26 oncogene homolog 1, Genbank Accession No.J04101) as a criteria.

[0083] First, mononuclear cells were isolated from the peripheral blood of the patient, and pure RNA was extracted by acid-phenol extraction. The RNA was treated according to the protocol provided by Affimetrix, and cDNA, dDNA and cRNA were prepared, then they were fragmented and hybridized with a DNA chip (U34Human). The amount of mRNA for ETS-1 protein (GenBank Accession No.J04101) was determined, and the result was represented by the ratio relative to the median obtained from all genes that gave significant signals on the DNA chip for normalization of the results.

[0084] From the data listed in Tables 4 and 5, the distributions on the control group and the patient group are as follows;

(1) Distribution of control group (N = 9): Average 3.05; Standard deviation 1.70; 5% lower limit threshold, 0.33

(2) Distribution of patient groups (N = 17): Average 0.72; Standard deviation 1.04; 5% upper limit threshold 2.38; 1% upper limit threshold 3.13

[0085] As seen from Tables 3 and 4, the values of the total 26 samples actually used for the study were; (C1) 0.37, (C2) 4.55, (C3) 1.09, (C4) 4.95, (C5) 3.33, (C6) 5.32, (C7) 2.83, (C8) 2.96, (C9) 2.05, (N1) 1.57, (N2) 3.16, (N3) 0.00, (N4) 2.45, (N5) 0.12, (S1) 0.00, (S2) 0.00, (S3) 0.24, (S4) 0.00, (S5) 0.00, (S6) 0.91, (S7) 0.00, (S8) 0.00, (S9) 2.30, (S10) 1.15, (S11) 0.00, and (S12) 0.36. The values from four

subjects (C2, C4, C5 and C6) were higher than the 1% upper limit threshold of the distribution of the patient group (3.13), and they were determined to be not included in the schizophrenic group at 99% reliability. That is, they were judged to be "normal."

[0086] In contrast, the expression levels of the gene obtained from ten patients (N3, N5, S1, S2, S3, S4, S5, S7, S8 and S11) were lower than the 5% lower limit threshold of the distribution of the control group (0.33). Therefore, they were determined to be not included in the non-schizophrenic (normal) group at 95% reliability. That is, they were determined to be "abnormal." As to the remaining 12 subjects, diagnosis could not be made on them. Therefore, as long as the above criteria were used, false diagnosis such as taking a subject included in the control group as "abnormal" or taking a subject included in the patient groups as "normal" could be avoided.

Example 2: Combined determination

[0087] In this Example, description will be given on diagnostic method with higher accuracy. In this method, mononuclear cells from peripheral blood of a patient were used, and the expression level of one gene was determined; i.e. mRNA for erythroblastosis virus oncogene homolog 1 (ETS-1) protein (v-ets avian erythroblastosis virus E26 oncogene homolog 1, GenBank Accession No.J04101), and the expression level was combined with the expression levels of two genes; i.e. gene for KIAA1048 protein (GenBank Accession No.AB028971) and gene for NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2497327 3' similar to SW:RHOD_HUMAN 000212 RHO-RELATED GTP-BINDING PROTEIN RHOD (Genbank Accession No.AW003733).

[0088] As described in Example 1, DNA chips (U95A, version 2) were used to assay the expression level of the gene for KIAA1048 protein (GenBank Accession No.AB028971). The 1% lower threshold of gene for KIAA1048 protein (GenBank Accession No.AB028971) was 0.66 for the distribution of the control group and the 1% upper threshold of the gene was 1.80 for the distribution of the patient group, and the level of the sample normalized by median was compared with these thresholds, thereby singular determination can be performed in the same manner as Example 1. In the total of 26 samples shown in Tables 4 and 5 (sum of groups C, N and S), no sample had the value of higher than 1.80 and determined to be "normal". In contrast,

seven subjects (N2, N5, S1, S3, S4, S5 and S12) were determined to be “abnormal” for having the values of lower than 0.66. Therefore, as long as the above thresholds are used, false diagnosis such as taking a subject included in the control group as “abnormal” or taking a subject included in the patient groups as “normal” could be avoided.

[0089] The same singular determination as in Example 1 was performed on the gene for NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2497327 3’ similar to SW:RHOD_HUMAN 000212 RHO-RELATED GTP-BINDING PROTEIN RHOD (GenBank Accession No.AW003733). The 1% lower threshold of this gene was 0.25 for the distribution of the control group and the 5% upper threshold of the gene was 1.82 for the distribution of the patient group, then value from an unknown sample was normalized to the median and compared with these thresholds. In total of 26 samples shown in Tables 4 and 5 (sum of groups C, N and S), no sample had the value of higher than 1.82 and determined to be “normal”. In contrast, six subjects (S1, S5, S6, S7, S8 and S9) were determined to be “abnormal” for having the value of lower than 0.25. Therefore, as long as the above thresholds are used, false diagnosis such as taking a subject included in the control group as “abnormal” or taking a subject included in the patient groups as “normal” could be avoided.

[0090] Using the expression levels on gene for EST-1 protein (GeneBank Accession No.J04101), tKIAA1048 protein (GenBank Accession No.AB028971) and NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2497327 3’ similar to SW:RHOD_HUMAN 000212 RHO-RELATED GTP-BINDING PROTEIN RHOD (GenBank Accession No.AW003733), determinations based on these three genes were combined and any of these determinations was taken as the final determination. As the result, in total of 9 subjects in the normal group, four subjects (C2, C4, C5 and C6) were determined to be “normal.” In the patient group, 14 subjects in 17 subjects were determined to be “abnormal,” except for three subjects (N1, N4 and S10). Therefore, as long as the above criteria are used, false diagnosis such as taking the control group as “abnormal” or taking the patient groups as “normal” could be avoided.

[0091] Thus, if the amount of expression in any gene exhibits significant difference (even in a singular gene), a patient can be diagnosed to be schizophrenic. Therefore, diagnosis of schizophrenia can be achieved with higher accuracy.

Example 3: Comprehensive probability determination

[0092] In this Example, the description will be given about an attempt to provide a more reliable method for diagnosis of schizophrenia. In this Example, plural genes exhibiting abnormal expression level (not within the normal range) in the patient group were by combined and statistical distributions of the expression levels of the genes in the normal control group were taken the into account.

[0093] In this Example, the distributions and deviations of the expression levels in normal subjects (unit; the ratio to the median of the DNA chips) are represented for the following 11 genes. Incidentally, since the expression levels of following three genes: 6) platelet-activating factor receptor (GenBank Accession No.D10202), 7) neuregulin 1 isoform HRG-alpha (GenBank Accession No.L41827), and 8) cytosolic component of the nuclear factor of activated T cells (GenBank Accession No.U08015) increased accompanied with schizophrenia, the distributions of the reciprocal numbers were represented for the three genes. The averages, standard deviations, and thresholds of the expression levels in the normal control group as well as the data of samples obtained on the individual genes are represented in Tables 3 and 4.

1) inositol polyphosphate-4-phosphatase, type 1, isoform b (GenBank Accession No.U26398), average ± standard deviation = 1.39 ± 0.47

2) v-ets avian erythroblastosis virus E26 oncogene homolog 1 (GenBank Accession No.J04101), average ± standard deviation = 3.05 ± 1.70

3) NCI_CGAP_Pr28 Homo sapiens cDNA clone IMAGE:2489058 3' similar to TR:Q15810 Q15810 CLONE 137308 ORF1 (GenBank Accession No.AW006742), average ± standard deviation = 1.50 ± 0.41

4) Source: Homo sapiens mRNA for KIAA1048 protein, complete cds, KIAA1048 protein (GenBank Accession No.AB028971), average ± standard deviation = 1.22 ± 0.24

5) NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2497327 3' similar to SW:RHOD_HUMAN 000212 RHO-RELATED GTP-BINDING PROTEIN RHOD (GenBank Accession No.AW003733), average ± standard deviation = 1.08 ± 0.36

6) platelet-activating factor receptor (GenBank Accession No.D10202), average ± standard deviation = 1.73 ± 3.75

7) neuregulin 1 isoform HRG-alpha (GenBank Accession No.L41827), average ± standard deviation = 1.43 ± 2.22

8) cytosolic component of the nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1 (GenBank Accession No.U08015), average ± standard deviation = 1.24 ± 0.69

9) Homo sapiens mRNA for KIAA1050 protein (GenBank Accession No.AB028973), average ± standard deviation = 0.73 ± 0.47

10) Homo sapiens cDNA clone DKFZp564J2262 (GenBank Accession No.AL036554), average ± standard deviation = 1.26 ± 0.82

11) Angelman Syndrome gene, E6-AP ubiquitin protein ligase 3A (GenBank Accession No.AF002224), average ± standard deviation = 9.96 ± 11.12

[0094] As described in Tables 4 and 5, +1 is given if the amount of gene expression to be tested is higher than the amount of average expression in the normal control, whereas –1 is given if the amount of expression is lower than that in the normal control. For example, in the case of N1, +1 is given on the nine genes of 1), 2), 4), 6), 7), 8), 9), 10) and 11) to give the score of +9, -1 is given on two genes of 3) and 5) to give the score of –2, therefore the total score results in +7. Moreover, in the case of N2, +1 is given on the six genes of 2), 3), 5), 6), 8) and 11) to give the score of +6, -1 is given on five genes of 1), 4), 7), 9) and 10) to give the score of –5, therefore the total score results in +1.

[0095] As described above, the comparison and the addition of the scores were performed on the 11 genes described above. In the case the total score of a subject was -8 or lower, the subject was determined to be "suffering from schizophrenia (abnormal)" ($P < (1/2)^8 < 0.004$), whereas in the case the score was between -5 and -7 ($P = 0.03$ to 0.008), the subject was determined to be "pseudo-positive (suspected to be abnormal)." As a result, among 17 patients, it was found that 11 patients (N3, N4, N5, S1, S2, S3, S4, S5, S7, S8 and S9) were determined to be "positive for schizophrenia (abnormal)", 4 patients (N1, S6, S11 and S12) were determined to be "pseudo-positive for schizophrenia (suspected to be abnormal)", and two patients (N2, S10) were determined to be "not confirmed", respectively. Thus, it was demonstrated that a diagnosis could be made on a subject as to whether the subject is suffering from schizophrenia or not with high reliably. To achieve it, the expression levels (including the distribution and the variance) of plural genes were compared with those of the normal control, and the multiplicative values of the probabilities were calculated respectively. As seen from above, simultaneous

measurement on the levels of gene expression can be achieved for many sample, use of DNA chips is advantageous.

Example 4: Linear discriminant analysis

[0096] In this Example, plural genes exhibiting altered expression between the chronic patient group and the acute patient group were used, and the values measured on the chronic and acute patients were treated by weighted linear discriminant, thereby diagnosis of schizophrenia was achieved with higher reliability.

The explanation will be made on the Example described in Table 6.

[0097] Table 6

		Discriminant score Y
Healthy subject	C1	8.87
Healthy subject	C2	15.02
Healthy subject	C3	3.54
Healthy subject	C4	12.05
Healthy subject	C5	13.00
Healthy subject	C6	11.19
Healthy subject	C7	12.67
Healthy subject	C8	7.07
Healthy subject	C9	7.98
Acute patient	N1	−2.80
Acute patient	N2	−8.65
Acute patient	N3	−10.74
Acute patient	N4	−11.45
Acute patient	N5	−12.55
Chronic patient	S1	−12.81
Chronic patient	S2	−5.24
Chronic patient	S3	−5.12
Chronic patient	S4	−16.51
Chronic patient	S5	−11.67
Chronic patient	S6	−10.66
Chronic patient	S7	−4.67
Chronic patient	S8	−4.52
Chronic patient	S9	−6.30
Chronic patient	S10	−17.77
Chronic patient	S11	−11.84
Chronic patient	S12	−19.31

[0098] In this Example, the expression levels of mRNAs of the following genes were measured using the mononuclear cells in peripheral blood derived from a test subject, that is, seven genes (genes 1, 2, 3, 7 and 9) exhibiting altered expression by acute schizophrenia; three genes (genes 4, 8 and 10) exhibiting altered expression by chronic schizophrenia; and two genes (genes 5 and 6), exhibiting altered expression by transition from acute phase to chronic phase of schizophrenia.

1) inositol polyphosphate-4-phosphatase, type 1, isoform b (GenBank Accession No.U26398) The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X1.

2) v-ets avian erythroblastosis virus E26 oncogene homolog 1 (GenBank Accession No.J04101). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X2.

3) NCI_CGAP_Pr28 Homo sapiens cDNA clone IMAGE:2489058 3' similar to TR:Q15810 Q15810 CLONE 137308 ORF1; ESTwr 28gIO X1 (GenBank Accession No.AW006742). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X3.

4) Source—Homo sapiens mRNA for KIAA1048 protein, complete cds, KIAA1048 protein (GenBank Accession No.AB028971). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X4.

5) NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2497327 3' similar to SW:RHOD_HUMAN 000212 RHO-RELATED GTP-BINDING PROTEIN RHOD (GenBank Accession No.AW003733). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X5.

6) Homo sapiens cDNA clone DKFZp564J2262 (GenBank Accession No.AL036554). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X6.

7) Angelman Syndrome gene, E6-AP ubiquitin protein ligase 3A (GenBank Accession No.AF002224). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X7.

8) neuregulin 1 isoform HRG-alpha (GenBank Accession No.L41827). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X8.

9) cytosolic component of the nuclear factor of activated T cells (GenBank

Accession No.U08015). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X9.

10) Homo sapiens mRNA for KIAA1050 protein (GenBank Accession No.AB028973). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X10.

[0099] The expression levels of above-mentioned genes represented by parameters X1 to X10 were used to calculate a discriminant score Y according to the following formula (Formula 1), which was optimized by linear discriminant analysis in advance.

[Formula 1]

$$Y = 9.35x(X1) - 0.15x(X2) + 7.50x(X3) + 8.46x(X4) + 0.99x(X5) - 0.66x(X6) - 0.42x(X7) - 5.59x(X8) - 3.17x(X9) - 1.89x(X10) - 13.03.$$

[0100] As an exemplary trail, the discriminant score Y was calculated for the 26 samples used in the study.

(1) Y = 8.87 for normal subject C1; 15.02 for normal subject C2; 3.54 for normal subject C3; 12.0 for normal subject C4; 13.00 for normal subject C5; 11.19 for normal subject C6; 12.67 for normal subject C7; 7.07 for normal subject C8; and 7.98 for normal subject C9.

(2) Y = -2.80 for acute patient N1; -8.65 for acute patient N2; -10.74 for acute patient N3; -11.45 for acute patient N4; and -12.55 for acute patient N5.

(3) Y = -12.81 for chronic patient S1; -5.24 for chronic patient S2; -5.12 for chronic patient S3; -16.51 for chronic patient S4; -11.67 for chronic patient S5; -10.66 for chronic patient S6; -4.67 for chronic patient S7; -4.52 for chronic patient S8; -6.30 for chronic patient S9; -17.77 for chronic patient S10; -11.84 for chronic patient S11; and -19.31 for chronic patient S12.

[0101] Then in the case the discriminant score Y used as a criteria gave a negative value in a subject, the subject is determined to be schizophrenic; while if discriminant score Y gave a positive value, the subject is determined to be not schizophrenic. Thus, expression levels on plural genes (including those exhibiting altered expression by transition from acute phase to chronic phase of schizophrenia) were measured and the results were treated by weighted linear discriminant analysis. Thereby, diagnosis on whether or not a subject is suffering from schizophrenia could be achieved by with high reliability.

Example 5: Mahalanobis discriminant analysis

[0102] In this Example, plural genes exhibiting altered expression between the chronic patient group and the acute patient group were used, and the individual expression levels measured on the chronic and acute patients were treated by linear addition with different weights by two manners and the obtained results were plotted in two dimensional coordinate system. Using this method the acute phase and the chronic phase of a patient could be distinguished at once. The explanation will be made using Table 7 and Figure 1.

[0103] Table 7

Caronical discriminant function coefficient

	Function	
	1	2
X1	1.887	−.842
X2	.135	.604
X3	1.652	−.179
X4	1.380	−1.930
X5	.581	1.272
X6	−.092	−.284
X7	−.091	.016
X8	−.853	1.489
X9	−.967	−.887
X10	−.090	1.215
(constant)	−2.467	−.783

non standarized coefficient

[0104] In this Embodiment, the expression levels of mRNAs of the following genes were measured using the mononuclear cells in peripheral blood derived from a test subject, that is, seven genes (genes 1, 2, 3, 7 and 9) exhibiting altered expression by acute schizophrenia; three genes (genes 4, 8 and 10) exhibiting altered expression by chronic schizophrenia; and two genes (genes 5 and 6) exhibiting altered expression by transition from acute phase to chronic phase of schizophrenia.

1) inositol polyphosphate-4-phosphatase, type 1, isoform b (GenBank Accession No.U26398) The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X1.

2) v-ets avian erythroblastosis virus E26 oncogene homolog 1 (GenBank Accession No.J04101). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X2.

3) NCI_CGAP_Pr28 Homo sapiens cDNA clone IMAGE:2489058 3' similar to TR:Q15810 Q15810 CLONE 137308 ORF1; ESTwr 28gIO X1 (GenBank Accession No.AW006742). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X3.

4) Source—Homo sapiens mRNA for KIAA1048 protein, complete cds, KIAA1048 protein (GenBank Accession No.AB028971). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X4.

5) NCI_CGAP_Kid11 Homo sapiens cDNA clone IMAGE:2497327 3' similar to SW:RHOD_HUMAN 000212 RHO-RELATED GTP-BINDING PROTEIN RHOD (GenBank Accession No.AW003733). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X5.

6) Homo sapiens cDNA clone DKFZp564J2262 (GenBank Accession No.AL036554). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X6.

7) Angelman Syndrome gene, E6-AP ubiquitin protein ligase 3A (GenBank Accession No.AF002224). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X7.

8) neuregulin 1 isoform HRG-alpha (GenBank Accession No.L41827). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X8.

9) cytosolic component of the nuclear factor of activated T cells (GenBank Accession No.U08015). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X9.

10) Homo sapiens mRNA for KIAA1050 protein (GenBank Accession No.AB028973). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X10.

[0105] The expression levels of above-mentioned genes represented by parameters X1 to X10 were used and the position of the test subject in a coordinate system (X,Y) was calculated according to the following formulas (Formula 2, Formula 3), which was optimized by linear discriminant analysis in advance.

[Formula 2]

$$X = 1.187x(X1) + 0.135x(X2) + 1.652x(X3) + 1.380x(X4) + 0.581x(X5) - 0.092x(X6) - 0.091x(X7) - 0.853x(X8) - 0.967x(X9) - 0.090x(X10) - 2.467$$

[Formula 3]

$$Y = -0.842x(X1) + 0.604x(X2) - 0.179x(X3) - 1.930x(X4) + 1.272x(X5) - 0.284x(X6) + 0.016x(X7) + 1.489x(X8) - 0.887x(X9) + 1.215x(X10) - 0.783$$

[0106] As an exemplary trial, calculation was performed for the 26 samples used in the study. As the result, as shown in Fig.1, it was revealed that the plots from each groups, i.e. the normal subject group, the acute schizophrenic patient group and the chronic schizophrenic patient group, gathered at certain regions of the coordinate system. Therefore, by adopting following criteria, determination of "acute schizophrenia," "chronic schizophrenia," and "non-schizophrenia" could be made for all of the samples. Meanwhile, those in other region could not be determined.

$-1.5 < X < 1$ & $1.1 < Y < 5$: acute schizophrenia

$-4 < X < 0$ & $-3 < Y < 1.1$: chronic schizophrenia

$1 < X < 4$ & $-2 < Y < 2$: non-schizophrenia

[0107] Thus, the expression levels of plural genes (including those exhibiting altered expression at transition from acute phase to chronic phase of schizophrenia) were measured and results were treated by weighted linear addition according to plural manners. Thereby, diagnosis on whether or not a subject is suffering from schizophrenia could be achieved by with high reliability.

Example 6: Analysis by mRNA level of kinase/phosphatase

[0108] The genes represented in Table 1 were those exhibiting altered mRNA expression by schizophrenia in mononuclear cells of patients and the functions of the genes were classified. As the result, it was revealed that the largest part of the genes was identified to be kinase/phosphatase, which are responsible for phosphorylation or de-phosphorylation of moleculars such as proteins. From this phenomenon, it was suggested that there may be some abnormalities on phosphorylation state of the mononuclear cells in schizophrenia. Paying attention to this phenomenon, the inventors selected the sixteen genes involved in phosphorylation reactions (kinase/phosphatase) described below. Then mRNA level alteration was investigated for the genes in patients.

1) Ndr serine threonine protein kinase (GenBank Accession No.Z35102).

The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X1.

inositol polyphosphate-4-phosphatase, type 1, isoform b (GenBank Accession No.U26398). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X2.

2) janus kinase 1 (GenBank Accession No.M64174). Its mRNA level on the gene chip based on the corrected median is represented by parameter X2.

3) inositol polyphosphate-4-phosphatase, type 1, isoform b (GenBank Accession No.U96919). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X3.

4) protein kinase, AMP-activated, alpha 1 catalytic subunit (GenBank Accession No.AB022017). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X4.

5) protein kinase C, nu (GenBank Accession No.AB015982). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X5.

6) MEK kinase (GenBank Accession No.U29671). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X6.

7) tyrosine kinase (GenBank Accession No.U07794). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X7.

8) serine/threonine-protein kinase PRP4 homolog (GenBank Accession No.U48736). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X8.

9) ribosomal protein S6 kinase, 90kD, polypeptide 3 (GenBank Accession No.U08316). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X9.

10) SNF1-like protein kinase (GenBank Accession No.U57452). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X10.

11) protein-tyrosine phosphatase (GenBank Accession No.D13540). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X11.

12) interferon-inducible RNA-dependent protein kinase (GenBank Accession No.U50648). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X12.

13) protein kinase, cAMP-dependent, catalytic, alpha (GenBank Accession No.X07767). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X13.

14) PCTAIRE protein kinase 1 (GenBank Accession No.X66363). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X14.

15) branched chain alpha-ketoacid dehydrogenase kinase (GenBank Accession No.AF026548). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X15.

16) phosphomevalonate kinase (GenBank Accession No.L77213). The mRNA level of the gene on the Gene Chip calibrated by median was represented by parameter X16.

[0109] In this Example, linear discriminant analysis was performed on an acute patient group including six patients (N1-N6), chronic patient group including 12 patients (S1-S12), and normal group including 12 normal subjects (C1-C12). Then alteration of the mRNA level was determined on the above-mentioned enzymes. In concrete, the mRNA signal intensities of the genes on the Gene Chip were assigned as parameters X1 to X16, and a discriminant score Y was calculated according to the following formula (Formula 4), which was optimized by linear discriminant analysis in advance.

[Formula 4]

$$Y = -4.60X1 - 5.77X2 + 7.74X3 - 13.9X4 + 24.2X5 + 4.11X6 + 3.20X7 - 7.41X8 + 5.76X9 - 1.06X10 + 34.1X11 + 0.15X12 + 4.16X13 - 2.51X14 + 14.9X15 - 2.85X16 + 3.50$$

[0110] The results are shown in Table 8 and Fig. 2. As seen from Fig. 2, the mRNA levels of the genes encoding the enzymes responsible for phosphorylation of mononuclear cells were altered in schizophrenic patients (S1-S12 and N1-N6). As the result, mRNA levels in schizophrenic patients were apparently distinguishable from those of normal subjects (C1-C12).

[0111] Table 8

Subject	Discriminant score
C1	−3.1574
C10	−3.84504
C11	−5.49943
C12	−4.73543
C2	−4.90849
C3	−3.73479
C4	−5.41805
C5	−4.27379
C6	−5.45122
C7	−3.9983
C8	−4.78686
C9	−4.95949
N1	1.8629
N2	3.14171
N3.	3.47818
N4	2.33664
N5	3.04534
N6	1.88635
S1	3.14728
S10	4.12939
S11	5.0998
S12	1.68138
S2	4.01308
S3	2.60176
S4	4.45117
S5	2.4072
S6	1.22822
S7	2.21729
S8	4.90192
S9	3.13867

	Mean value	S.D.
C	-4.56402	0.758766
N	2.625187	0.690093
S	3.25143	1.269113

Example 7: Investigations on patients with other psychiatric diseases

[0112] As to patients suffering from depression or panic syndrome (B1-B6) which may exhibit symptoms similar to schizophrenia, using the linear discriminant (Formula 4) shown in the Example 6, gene expression profiling was performed on genes of mononuclear cells in blood of the patients, using the DNA Chips by the

method as described above. In the same manner as in Example 6, the mRNA signal intensities encoding 16 enzymes involved in phosphorylation reaction were applied to the same formula (Formula 4) used in Example 6 to provide a discriminant score Y. The results are shown in Table 9.

[0113] As shown in Table 9, in five patients except for patient B3, all of the discriminant scores were negative values, thus the patients were determined to be not suffering from schizophrenia. As to the patient B3, the Y score was +1.6 and exhibited weak positive. The result of this biological test may suggest the possibility that the patient B3 is suffering from schizophrenia. As seen from above, the discriminant according to Formula 4, paying attention to the mRNA levels of the genes encoding kinases/phosphatases, may be useful for determination of psychiatric diseases including schizophrenia.

[0114] Table 9

Subject	Discrimant score
B1	−4.37615
B2	−4.96375
B3	1.60492
B4	−4.34437
B5	−2.23161
B6	−1.29941

Example 8: Mahalanobis discriminant analysis

[0115] In this Example, the mRNA intensities of the genes encoding the enzymes involved in phosphorylation or de-phosphorylation (kinases/phosphatases) were measured like Example 6 and two primary discriminant functions were determined using Mahalanobis cluster analysis. The discriminant scores of each sample were plotted on a two-dimensional plane, then the results were discriminated into three groups, i.e. acute patient group (N1-N6), chronic patient group (S1-S12) and normal group (C1-C12). The mRNA expression intensities (X1 to X16) were obtained for the 16 genes in the Example 6 on a DNA Chip, and the results were applied to the following two linear equations (Formula 5 and Formula 6).
The coordinate consisted of (X, Y) in the Table 10 and in the Table 11, and the positions in the coordinate were calculated for the test subjects. The results from the schizophrenic patients (S1-S12 and N1-N6: the same as in Table 8) and those

from the normal subjects (C1-C12) were shown in Table 10. Moreover, the results from the six patients (B1-B6: the same as in Table 9) suffering from psychiatric diseases other than schizophrenia were shown in Table 11. The results shown in Table 10 and Table 11 are plotted on a two-dimensional discrimination diagram shown in Fig.3. In Fig.3, the solid column represents the results from chronic schizophrenic patients, the open column represents those from acute schizophrenic patients, and the hatched column represents those from normal subjects.

[Formula 5]

$$X = -4.32X1 - 6.96X2 - 0.31X3 - 12.4X4 + 14.4X5 + 4.84X6 + 2.97X7 - 21.5X8 + 8.11X9 + 8.30X10 + 46.8X11 - 0.89X12 + 4.26X13 - 2.48X14 + 16.7X15 - 4.97X16 + 7.03$$

[Formula 6]

$$Y = -1.96X1 - 0.30X2 + 13.8X3 - 6.74X4 + 21.8X5 + 0.37X6 + 1.42X7 + 17.1X8 - 1.31X9 - 13.4X10 - 6.13X11 + 1.50X12 + 1.26X13 - 0.90X14 + 2.48X15 + 1.99X16 - 3.72$$

[0116] Table 10

Subject	Discrimant score X	Discrimant score Y
C1	−3.31117	−0.86085
C10	−5.52444	1.02784
C11	−6.2157	−0.87543
C12	−5.15963	−1.02175
C2	−5.15608	−1.3264
C3	−4.24532	−0.561
C4	−6.69962	−0.06117
C5	−4.37362	−1.31594
C6	−5.35035	−1.996
C7	−5.77044	1.10472
C8	−5.55691	−0.55805
C9	−4.76531	−1.95843
N1	0.04866	3.15851
N2	0.48305	4.76873
N3.	1.74519	3.59529
N4	0.44795	3.42336
N5	1.44364	3.26529
N6	0.46093	2.62548
S1	4.27598	−0.49912
S10	6.49481	−1.88558
S11	5.64561	0.97659
S12	3.5928	−2.08721
S2	5.83667	−1.17126
S3	4.23527	−1.38722
S4	6.12612	−0.81531
S5	5.07678	−2.89504
S6	2.49265	−1.34124
S7	3.75289	−1.38186
S8	5.50818	0.8251
S9	4.46141	−0.77205

[0117] Table 11

	Discrimant score X	Discrimant score Y
B1	−6.84479	1.94521
B2	−6.09437	−0.11655
B3	0.93432	1.47941
B4	−2.07503	−4.63643
B5	−4.18643	1.96029
B6	−1.44387	−0.24133

[0118] As shown by the sets of circles shown in Fig. 3, the scores from "acute schizophrenia group" "chronic schizophrenia group" and "normal group" gathered in a certain region of the graph, therefore, the three groups were completely distinguishable from each other. In addition, the blind data were obtained from the group of patients suffering from psychiatric diseases other than schizophrenia (B1-B6) and the data were also treated by the same Formulas (Formula 5 and Formula 6). The results were plotted in the same figure by the "X" marks, to reveal that the patients do not belong to the regions corresponding to the "acute schizophrenia group" "chronic schizophrenia group" and "normal group" described above. This suggests that the discriminant is also useful for the discrimination of psychiatric diseases other than schizophrenia.

[0119] The method of the invention provides a group of genes useful for objective diagnosis on whether or not a test subject is suffering from schizophrenia in non-invasive manner. Therefore, development of a DNA chip containing genes effective for diagnosis of schizophrenia may be achieved utilizing the knowledge obtained in this invention.